ASSESSMENT OF MICROBIAL LOADS PRESENT IN TWO WESTERN CAPE RIVERS USED FOR IRRIGATION OF VEGETABLES

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

Agriculture in the Western Cape is not only one of the most important economic sectors but also provides many job opportunities. Over the last few years the sustainability of this successful industry has become threatened by the faecal pollution of rivers used to irrigate produce that will be consumed raw or after minimal processing. This situation not only poses an enormous risk to the health of the consumer but also to farmers who stand to lose their export licenses.

The purpose of this study was to determine the microbial types and loads in river water, irrigation water and on irrigated produce. A baseline study was done on four sites in two Western Cape rivers. These sites were chosen to allow for the sampling of river water, irrigation water and irrigated produce so as to determine whether a link between the use of contaminated irrigation water and the microbial population found on irrigated produce exists.

The physico-chemical analyses used in the study consisted of: pH, alkalinity, water temperature, conductivity and chemical oxygen demand. The microbial monitoring included the aerobic colony counts (ACC) and the enumeration of the total coliforms, faecal coliforms, staphylococci, enterococci, and aerobic and anaerobic sporeformers present in the water samples. The presence or absence of the potential pathogens like *E. coli*, *Listeria* and *Salmonella*, was also determined.

During the baseline study faecal coliform counts as high as 160 000 organisms.100 mL^{-1} were noted in the Plankenburg River, while counts as high as 460 000 organisms.100 mL^{-1} were found in the Mosselbank River. Apart from this, high numbers of staphylococci and intestinal enterococci were often found, while *E. coli*, *Listeria* and *Salmonella* were present in samples from both of these rivers.

Based on the results of the baseline study on the two rivers it was decided to do a more intensive study on the microbial load of the river and irrigation water as well as irrigated produce from the Mosselbank site. Lettuce and cabbages from a commercial farmer's fields were chosen as the irrigated produce. During the warmer summer months, ACC counts in the river samples peaked at 12 8000 000 $cfu.mL^{-1}$, while faecal coliform counts of 1 600 000 organisms.100 mL^{-1} were found. The three potential pathogens (*E. coli*, *Listeria* and *Salmonella)* were present in all the river samples taken during this period. While the counts of indicator bacteria in the irrigation water was often lower, faecal coliform counts as high as 1 600 000 organisms.100 mL^{-1} and several other potential pathogens were found on the irrigated lettuce and cabbage. This could indicate a possible

"build-up" of contamination on the produce with the repeated application of the tainted irrigation water.

According to guidelines published by DWAF in 2008, water to be used for irrigation should not contain more than 4 000 organisms.100 mL^{-1} faecal coliforms if it is used for the irrigation of crops that are to be consumed raw or after a minimal processing step, as this would increase the health risk to the consumer. Guidelines published by the South African Department of Health are even stricter and state that raw vegetables and fruit should not contain more than 200 coliform organisms per gram, while *E. coli* and *L. monocytogenes* should be absent in one gram, and *Salmonella spp*. in 25 grams of the produce, respectively. From the data obtained during this study it was evident that the two rivers monitored regularly contained faecal indicators at levels much higher than those proposed in national and international guidelines for safe irrigation, making them unfit for the irrigation of MPF's.

It could be concluded that the rivers investigated during this study contained high levels of faecal contamination. Since some of the pathogens isolated from the river and irrigation water and the irrigated produce, it suggests a carry-over of microbial contamination from the river water to the irrigated produce. This was, however, only done using the traditional international methods and the presence of specific pathogens should in future be confirmed by means of molecular techniques.

UITTREKSEL

Landbou is nie net die een van die belangrikste ekonomiese sektore in die Wes-Kaap nie, maar verskaf ook vele werksgeleenthede. Oor die afgelope paar jaar word die volhoubaarheid van hierdie suksesvolle industrie egter bedreig deur die fekale kontaminasie van riviere wat gebruik word vir die besproeiing van voedsel wat rou of na ‘n minimale prosesserings stap ingeneem word. Hierdie situasie hou nie net ‘n groot gevaar vir die gesondheid van verbruikers in nie, maar ook vir boere wat hul uitvoerlisensies hierdeur kan verloor.

Die doel van hierdie studie was om die ladings en tipes mikrobes in rivier water, besproeiingswater en op besproeide produkte vas te stel. ‘n Basiese studie van vier liggings in twee Wes-Kaapse riviere is gedoen. Hierdie liggings is só gekies dat dit moontlik was om die rivier water, besproeiingswater en die besproeide produkte te monitor, en daar sodoende vasgestel kon word of daar ‘n verhouding is tussen die gebruik van gekontamineerde besproeiingswater en die mikrobe populasie wat op die besproeide produkte aanwesig was.

Die fisiko-chemiese analises wat gedurende die studie gedoen is, het pH, alkaliniteit, water temperatuur, geleidingsvermoë en die chemiese suurstof vereiste (COD) ingesluit. Die mikrobiese analises het die aërobe kolonie tellings (ACC) en die enumerasie van die totale kolivorme, fekale kolivorme, staphylococci, enterococci en die aërobe en anaërobe spoorvormers ingesluit. Daar is ook vir die aanwesigheid van potensiële patogene soos *E. coli*, *Listeria* en *Salmonella* getoets.

Gedurende die basiese studie is fekale kolovorme tellings van so hoog as 160 000 organismes.100mL^{-1} in die Plankenburg Rivier aangeteken, terwyl tellings van so hoog as 460 000 organismes.100mL^{-1} in die Mosselbank Rivier gevind is. Hoë tellings stafielokokki en intestinale enterokokki is gereeld genoteer, terwyl *E.coli*, *Listeria* en *Salmonella* uit die waters van beide hierdie riviere geïsoleer is.

Gebaseer op hierdie resultate is daar besluit om ‘n meer intensiewe studie van die rivier, besproeiingswater en die besproeide produkte van die Mosselbank Rivier te doen. Blaarslaai en kool van ‘n kommersiële boer se lande is vir hierdie doel gekies. Gedurende die warmer somer maande het die aërobe kolonie tellings in die rivier ‘n piek van 12 800 000 kve.mL^{-1} bereik, terwyl fekale kolivorme tellings van 1 600 000 organismes.100mL^{-1} genoteer is. Die drie potensiële patogene (*E. coli*, *Listeria* en *Salmonella*) was aanwesig in al die monsters wat gedurende hierdie tydperk van die rivierwater geneem is. Alhoewel die tellings indikator bakterieë in die besproeiingswater meestal laag was, is tellings fekale kolivorme van so hoog as 1 600 000 kve.100mL^{-1} en verskeie ander potensiële patogene

op die besproeide blaarslaai en kool gevind. Dit kan dui op ‘n moontlike opbou van kontaminasie op die produkte met die herhaalde besproeiing met gekontamineerde besproeiingswater.

Volgens die riglyne wat in 2008 deur DWAF gepubliseer is, mag water wat vir die besproeiing van minimaal geprosesseerdevoedsels gebruik word nie meer as 4 000 organismes.100mL^{-1} bevat nie, aangesien dit die gesondheid van die gebruiker in gevaar mag stel. Die riglyne van die Suid-Afrikaanse Departement van Gesondheid is selfs strenger en beveel aan dat rou vrugte en groente nie meer as 200 kolivorme en geen *L. monocytogenes* per gram, en geen *Salmonella spp*. in 25 g van die produk mag bevat nie. Vanuit die data wat tydens hierdie studie ingesamel is, is dit duidelik dat die twee riviere gereeld fekale indikators bevat het teen vlakke baie hoër as wat in die nasionale en internasionale riglyne aanbeveel word. Hierdie water is dus nie geskik vir die besproeiing van minimaal geprosesseerde produkte nie.

Die afleiding kan gemaak word dat die riviere wat tydens hierdie studie gemonitor is, hoë vlakke van fekale kontaminasie bevat het. Aangesien sommige van die patogene vanuit beide die rivier- en besproeiingswater, en vanaf die besproeide produkte geïsoleer is, kan dit dui op ‘n moontlike oordrag van mikrobiese kontaminasie vanuit die rivierwater na die besproeide produkte. Tydens hierdie studie is daar egter net van die tradisionele internasionale metodes gebruik gemaak. Vir toekomstige navorsing word dit aanbeveel dat die aanwesigheid van die spesifieke patogene deur die gebruik van molekulêre metodes bevestig word.

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CONTENTS

Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1
INTRODUCTION

Since 1994 the South African population has undergone major demographic changes (Venter *et al*., 2007). With an annual population growth rate of 3.34% experienced since 1975, the population has increased from 5.17 million in 1904 to 46.9 million in 2004. Thus, the population has increased by eightfold while the natural resources have remained unchanged (DEAT, 2006). With migration from rural areas taking place, it is estimated that 58% of the South African population currently live in urbanised areas, and of these 11.5% live in informal settlements where the provision and upkeep of sanitary services is rare (Stats SA, 2005; DEAT, 2006).

The Western Cape has a flourishing economy and together with the natural resources, many citizens come here with dreams of better circumstances and ample job opportunities (St. Louis & Hess, 2008), only to find that 25.5% of the 4.75 million inhabitants are currently unemployed (Stats SA, 2006; WESGRO, 2007). With no place to stay and no source of income, many of these individuals and their families are forced to seek shelter in informal settlements where the upkeep of sanitation is not seen as a priority. A total deficit of sanitary facilities is seen in many informal settlements throughout the Western Cape and the residents are inclined to use uninhabited land or buckets for these purposes. The contents of the buckets are discarded into nearby rivers or in solid waste containers, and as many of these settlements do not receive regular waste removal, this is an ideal breeding ground for pathogens (DEAT, 2006). During the rainfall season this waste is washed over the surfaces by the downpour and, apart from the health implications this may bring about for the residents, the contamination of nearby water sources becomes a problem (Barnes, 2003). In other “privileged” settlements where sanitary infrastructure is present, the residents far outnumber these facilities. Furthermore, the facilities are often filthy and not in proper working order (Britz *et al*., 2007) and therefore residents are forced to; once again, make use of buckets or uninhabited land (Okafu *et al*., 2003).

Many of the residents in these unserviced informal settlements use the water from nearby rivers or streams for all their domestic needs including cooking, washing and bathing. To alleviate the suffering in these areas, free basic water is allocated

(Barnes, 2003) but sadly no infrastructure is in place to redirect the waste to treatment plants for purification, and as a result large volumes end up in the nearest river, threatening the very sensitive river ecology (DEAT, 2006). With the growing number of HIV positive individuals present in our communities, especially in poverty stricken areas, contaminated water is becoming an even bigger problem as these individuals are more susceptible to infection than healthy individuals. Where others may only become ill, such an infection may be lethal for an infected individual (DEAT, 2006). An alarming 5.57 million South Africans are now infected with the virus and numbers are still growing. Authorities are encouraging malnourished individuals in poorer communities to grow their own vegetables as these products are a great source of the much needed macro and micro nutrients, but often the only water these communities have access to is a nearby river or stream (NFCS, 2000). With an already battered immune system and frail body, the consumption of these "tainted" vegetables might just be the final straw for these disease-plagued individuals.

South Africa is ranked amongst the top 20 countries exporting several agricultural products, and is seen as the continent leader where the export of fresh produce is concerned. Currently, 73% of Africa's fruit exported to the USA is grown within our borders (NDA, 2007; Britz *et al*., 2007). Of all the fruit grown in South Africa, 60% is intended for export to Europe, Vietnam and Malaysia, and of this percentage 55 to 60% is grown in the Western Cape (WESGRO, 2008). The favourable climatic conditions make the province the ideal environment for thriving agricultural production. With exports worth billions of Rands per year, the agricultural sector is also the major employer in this region. With more than 8 000 commercial farmers and hundreds of thousands of farm workers, the agricultural industry supports more than 1.5 million people in the Western Cape alone (WESGRO, 2006). On a national level the agricultural industry formally employs more than 9% of the South African population when direct evaluations (farmers and farm workers) are made (WESGRO, 2006).

South Africa is a semi-arid country where rainfall differs greatly between regions and from one year to another (NWRS, 2004). Irrigation is thus a necessity for the largest part of the country if the national and international demands for quality agricultural products are to be met. Although the National Water Resource Strategy (NWRS, 2004) states that enough water will be available to satisfy the demand in

years to come, the effect climate change will have has not been taken into consideration and several researchers have expressed concern that the increased rate of population growth and urbanisation will lead to excessive demands for fresh water and food (Spinks *et al*., 2006; Madungwe & Sakuringwa, 2007). With the Western Cape becoming warmer and drier because of climate change (Midgley *et al*., 2005), the competition for water between the industrial, urban and agricultural sectors will increase (Hamilton *et al*., 2006).

An estimated 67% of the country's fresh water is used for irrigation purposes. A third of this is used for the irrigation of fresh produce intended for export (Backeberg, 1996). South African farmers get irrigation water mainly from rivers, farm dams, reservoirs and groundwater supplies (Britz *et al*., 2007). Where infrastructure for the treatment of wastewater is lacking, the water from the abovementioned sources may become contaminated, but with irrigation water being one of the limiting factors in successful farming practices in arid and semi-arid countries, farmers are left with no other choice but to use water of sometimes questionable microbial quality (Gómez *et al*., 2006).

Data on the microbiological quality of the country's rivers is scarce, but the little data available paints an ominous picture. In 2004, counts of 560 000 000 *E.coli* organisms per 100 mL was found in the Plankenburg River near Stellenbosch (unpublished research data from Dr. J.M Barnes, University of Stellenbosch) and similar counts were found in many of the country's other major rivers. According to the World Health Organisation (1989) irrigation water containing more than 1 000 faecal coliforms per 100 mL water is seen as a serious risk for the spread of disease. As this organism is seen as an indicator of faecal contamination, many studies only report the count of *E. coli* present in the water while the presence of many other potential human pathogens remains unknown. Although faecal coliforms can be seen as the best available indicator of faecal pollution in water, they can originate from non-faecal sources (Savichtcheva & Okabe, 2006) and are often found when no other enteric pathogens are present.

Farmers along the Berg River received a warning from the European Union regarding the quality of their irrigation water in 2005 (Anon., 2005). Concern was raised about the effect the dire microbial state of the country's rivers might have on the agricultural export activities and, with that, the economy of the Western Cape and South Africa.

Yuk *et al*. (2006) reported a marked increase in the number of foodborne outbreaks globally. Although many reasons for this occurrence are listed, several researchers have reported faecally polluted irrigation water to be the source of contamination of the implicated products (Beuchat, 1996; Brackett, 1999; Okafu *et al*., 2003; WHO, 2004; Elizaquível & Aznar, 2008; Elviss *et al*., 2009). With no system in place where water- or foodborne outbreaks can be reported, data of outbreaks linked to fresh produce or contaminated water in South Africa is scarce (Britz *et al*., 2007). According to the WHO (2004) more than 80% of the 1.8 million fatal cases of diarrhoea are caused by unsafe water and a lack of sanitation and hygiene. This could be reduced by more than 30% if sanitation in rural areas was improved (WHO, 2004).

The impact that deteriorating river water quality may have on community health, food safety, export activities, the economic environment, and employment in the agricultural sector is far-reaching. This therefore necessitates a study be done to assess the extent of contamination in South African rivers, and whether the contamination of irrigation water really poses a threat to the assurance of food safety and the health of consumers.

The overall objective of this research is to do an exploratory study of the types and quantities of microbes present in selected river and irrigation waters. Produce irrigated with this water will subsequently be harvested and the organisms present identified and quantified using standard methods. With the data from the exploratory studies it will be endeavoured to establish the possibility of whether any direct microbial carry-over links exist between irrigation water and the fresh produce.

REFERENCES

Anonymous (2005). Berg River endangering health of economy. Cape Times, August 2, 2005.

Backeberg, G.R. (1996). Presidential Address: Constitutional change and natural resource policy reform in South Africa. *Agrekon*, **35** (4), 160-169.

Barnes, J.M. (2003). *The Impact of Water Pollution from Formal and Informal Urban Developments along the Plankenbrug River on Water Quality and Health Risk*. PhD, University of Stellenbosch, South Africa.

Barnes, J.M. (2007). University of Stellenbosch, Stellenbosch, South Africa. Unpublished data.

Beuchat, L. R. (1996). Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection*, **59**, 204–216.

Brackett, R.E. (1999). Incidence, contributing factors, and control of bacterial pathogens in produce. *Postharvest Biology and Technology*, **15**, 305-311.

Britz, T.J., Barnes, J.M., Buys, E.M., Ijabadeniyi, O.A., Minnaar, M., Potgieter, N., Sigge, G.O., Ackermann, A., Lötter, M., Taylor, M.B., van Zyl, W., Venter, I. & Netshikweta, R. (2007). Quantitative investigation into the link between irrigation water quality and food safety. *Water Research Commission Project Report K5/1773//4*, Pretoria, South Africa.

DEAT (Department of Environmental Affairs and Tourism). (2006). South Africa Environment Outlook. A report on the state of the environment. Department of Environmental Affairs and Tourism, Pretoria.

Elizaquível, P. & Aznar, R. (2008). Comparison of four commercial DNA etraction kits for PCR detection of *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7, and *Staphylococcus aureus* in fresh, minimally processed vegetables. *Journal of Food Protection*, **71** (10), 2110-2114.

Elviss, N.C., Little, C.L., Hucklesby, L., Sagoo, S., Surman-Lee, S., de Pinna, E. & Threlfall, E.J. (2009). Microbiological study of fresh herbs from retail premises uncovers an international outbreak of salmonellosis. *International Journal of Food Microbiology*, 2009.

Gómez, M., de la Rua, A., Garralón, G., Plaza, F., Hontoria, E. & Gómez, M.A. (2006). Urban wastewater disinfection by filtration technologies. *Desalination*, **190**, 16-28.

Hamilton, A.J., Stagnitti, F.S., Premier, R. & Boland, A.M. (2006). Is the risk of illness through consuming vegetables irrigated with reclaimed wastewater different for different population groups? *Water Science & Technology*, **54** (11-12), 379-386.

Madungwe, E. & Sakuringwa, S. (2007). Greywater reuse: A strategy for water demand management in Harare? *Physics and Chemistry of the Earth*, **32**, 1231-1236.

Midgley, G.F., Chapman, R.A., Hewitson, B. & Johnston, P. (2005). A status quo, vulnerability assessment of the physical and socio-economic effects of climate change in the Western Cape. Report to the Western Cape Government, Cape Town, South Africa. CSIR Report No. ENV-S-C 2005-703.

NDA (National Department of Agriculture). (2007). Agricultural Statistics. National Department of Agriculture, South Africa.

NFCS (National Food Consumption Survey. (2000). The National Food Consumptions Survey: Children aged 1-9 years, South Africa, 1999. Department of Health, South Africa.

NWRS (National Water Resource Strategy) (2004). First Edition, September 2004. Department of Water Affairs & Forestry, South Africa.

Okafu, C.N., Umoh,V.J. & Galadima, M. (2003). Occurrence of pathogens on vegetables harvested from soils irrigated with contaminated streams. *The Science of the Total Environment*, **311**, 49-56.

Savichtcheva, O. & Okabe, S. (2006). Alternative indicators of fecal pollution: Relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. *Water Research*, **40**, 2463-2476.

Spinks, A.T., Dunstan, R.H., Harrison, T., Coombes, P. & Kuczera, G. (2006). Thermal inactivation of waterborne pathogenic and indicator bacteria at sub-boiling temperatures. *Water Research*, **40**, 1326-1332.

St. Louis, M.E. & Hess, J.J. (2008). Climate Change: Impacts on and Implications for Global Health. *American Journal of Preventative Medicine*, **35** (5), 527-538.

Stats SA. (2005). General Household Survey – July 2005. [www document]. URL http://www.ukzn.ac.za/heard/resources/Stats/general%20household%20survey%20july%202005.pdf. 4 March 2009.

Stats SA. (2006). South African Statistics. [www document]. URL: http://www.ukzn.ac.za/heard/resources/Stats/SA%20Statistics%202006.pdf. 4 March 2009.

Velazquez, L.C., Barbini, N.B., Escudero, M.E., Estrada, C.L. & de Guzman, A.M.S. (2009). Evaluation of chlorine, benzalkonium chloride and lactic acid as sanitizers for reducing *Escherichia coli* O157:H7 and *Yersinia enterocolitica* on fresh vegetables. *Food Control*, **20**, 262–268.

Venter, S.N., Steynberg, M.C., de Wet, C.M., Hohls, D., du Plessis, G. & Kfir, R. (1997). A situational analysis of the microbial water quality in a peri-urban catchment in South Africa. *Water Science & Technology*, **35** (11-12), 119-124.

WESGRO (2006). Fruit processing sector brief. Western Cape Trade and Investment Promotion Agency. Pp. 36-40.

WESGRO (2007). WESGRO homepage. [WWW document]. URL http://www.wesgro.org.za. 22 June 2007.

WESGRO (2008). About SA. [www document]. URL: http://www.wesgro.org.za/inde.php?view=article&catid=34%3Ainvest-in-the-wc. 3 December 2008.

WHO (World Health Organisation) (1989). Health guidelines for the use of wastewater in agriculture and aquaculture. Geneva, Switzerland: World Health Organisation. Technical Report Series 778.

WHO (World Health Organisation) (2004). Water, Sanitation and Hygiene Links to Health – Facts and Figures updated November 2004. [WWW document]. URL http://www.who.int/water_sanitation_health/publications/facts2004/en/. 3 October 2008.

Yuk, H-Y., Yoo, M-Y., Yoon, J.W., Moon, K.D., Marshall, D.L. & Oh, D.H. (2006). Effect of combined ozone and organic acid treatment for control of Escherichia coli O157:H7 and Listeria monocytogenes on lettuce. *Journal of Food Science*, **71** (3), 83–87.

CHAPTER 2
LITERATURE REVIEW

WATER SITUATION IN SOUTH AFRICA

In South-Africa, surface water is mainly utilised for domestic, industrial and irrigation purposes. Only 8.6% of the annual rainfall is available as surface water and most of it flows over the country's slanted terrain towards the eastern parts (DEAT, 2006). The Western Cape is becoming warmer and drier because of climate change (Midgley *et al.,* 2005; St. Louis & Hess, 2008) – this while rivers are unable to provide ample water of a good microbial quality. In many populated areas of the world, water is already in short supply (Marcucci & Tognotti, 2002; Oweis & Hachum, 2009; Komnenic *et al*., 2009; Qiao *et al*., 2009, Malley *et al*., 2009), and rivalry between the industrial, urban and agricultural sectors is on the increase (Hamilton *et al*., 2006; Oweis & Hachum, 2009). For this reason many farmers are forced to use water of questionable microbial quality or even reclaimed wastewater to irrigate their crops (Parrot *et al*., 2008).

South Africa has experienced expansion of informal settlements due to demographic changes since 1994 (Venter *et al*., 1997; Tempelhoff, 2009). As most of these settlements do not have proper sanitary and water services, communities are forced to use nearby freshwater sources for all their domestic needs – including drinking water (Barnes, 2003; Tempelhoff, 2009). Not only does this increase the chances of infection, but downstream users are also at greater risk. Dilution and die-off are the only means of lowering the microbial load of a surface water source.

The release of the National Water Resource Strategy (NWRS, 2004) by the Department of Water Affairs and Forestry (DWAF), hopes to manage the demands and use of water by the different sectors effectively. According to this report, enough water of a satisfactory quality should be available to meet the future demands if the resources are managed strictly, but this strategy did not take the influence of climate change on the available resources into account. It is estimated that rainfall in the western parts of the country may decline with as much as 10% by 2015 while an increase of similar proportions will be seen in the eastern parts. It is also suggested that greater variation in the volume and intensity of rain downpours may be experienced (DEAT, 2006; St. Louis & Hess, 2008). The DEAT report further emphasised the poor condition of the country's aquatic ecosystems. More than 50% of our wetlands have been destroyed, only 26% of the rivers are still undamaged, and 54% of them are critically endangered (DEAT, 2006).

Several factors contribute to the condition of the country's rivers including pollution with domestic and sewage effluents, industrial effluents, runoff from informal settlements and the premeditated discharge or leakage of partially treated sewage from poorly maintained treatment works (Britz *et al*., 2007; Tempelhoff, 2009). According to the minister of Water Affairs and Forestry (NWRS, 2004), the degradation of the countries rivers can be directly linked to microbial contamination ending up in rivers because of malfunctioning water treatment plants. This can be seen as one of the major causes of disease in our country (NWRS, 2004; Tempelhoff, 2009).

It is extremely difficult to find data on the microbiological quality of South African rivers (Britz *et al*., 2007), but from the limited data available it is evident that the rivers and streams are in a dire state. It was reported that in 2003 counts as high as 13 million *E. coli* per 100 mL water was measured in the Jukskei River in Gauteng, and 1 080 000 organisms per 100 mL was found in the uMngeni River in KwaZulu Natal (unpublished research data from J.M. Barnes, University of Stellenbosch). In the Western Cape, reports on the Plankenburg River near Stellenbosch showed that microbial levels peaked at 560 000 000 *E. coli* organisms per 100 mL of water in 2004 (unpublished research data from J.M. Barnes, University of Stellenbosch). Two years later counts of 9 200 000 *E. coli* per 100 mL were still observed. Another major river in the province, the Berg River, had counts of 92 080 *E. coli* per 100 mL and 2 440 000 000 *E. coli* per 100 mL, respectively for a site in Franschhoek and one near an informal settlement in Paarl.

As much as 67% of the country's fresh water is used for irrigation purposes (Backeberg, 1996). Of this, 33% is used by farmers who are responsible for approximately 35% of the domestic foodstuffs and 85% of the total products meant for export. Since the use of this resource is so extensive, no other could take its place if it was to become polluted with heavy microbial loads. It is thus of great importance that everything be done to protect the quality of South Africa's fresh water resources.

With water scarcity on everyone's lips, the debate around the use of wastewater as an alternative source of irrigation is receiving more and more attention (Scott *et al.*, 2004). Although wastewater reuse is not a new concept to farmers around the world, new questions are arising concerning the health implications this may have for consumers (Scott *et al.*, 2004).

URBANISATION, INFORMAL SETTLEMENTS AND THE LACK OF SANITATION

Statistics for 2006 showed the growth of the Western Cape's GDP to be 5.9% higher than that experienced on a national level (WESGRO, 2008). Taking the abovementioned

prosperity of the province's economy and abundant natural resources into account, it is not surprising that more and more people come to the province seeking a better life for themselves and their families (WESGRO, 2008; St. Louis & Hess, 2008).

In 2006, it was estimated that 4.75 million of the country's citizens live in the Western Cape (Stats SA, 2006). Although there is still migration into the province, the growth rate is expected to decline over the next decade (WESGRO, 2007). Many people believe that the Western Cape offers a better quality of life and that there are ample job opportunities, while this may be true the unemployment rate is currently 25.5% (Stats SA, 2007) and numerous individuals end up without employment, a roof over their heads or food to eat and are forced to seek refuge in informal settlements. These settlements are more often than not situated near industrial areas (Barnes, 2003) where the upkeep of sanitation is not of the highest priority.

In recent years concern has been expressed that an increased rate of population growth will lead to excessive demands for fresh water (Spinks *et al*., 2006; Madungwe & Sakuringwa, 2007; Rietveld *et al*., 2009). Several authors estimated that by 2025 as much as two thirds of the global population will live in urban communities (Raschid, 2004; Campbell *et al*., 2008; Parrot *et al*., 2008; Parrot *et al*., 2009). Municipalities are already struggling to keep up with the increased demand for water and sanitation services because of the continuous migration and population growth and with the lack of these services in many informal settlements, vast volumes of human waste enter our rivers (Britz *et al*., 2007; Tempelhoff, 2009).

Around 58% of the South African population is estimated to live in urban areas with 11.5% of the households in informal settlements where the provision of basic services is a rare luxury (DEAT, 2006; Stats SA, 2005). In many informal settlements in the Western Cape there is an average of one toilet per 60 to 100 occupants (Britz *et al*., 2007). These are some of the "luckier" communities, as many other settlements have no sanitary facilities and 10.2% of households are forced to make use of uninhabited land or buckets for these purposes (Barnes, 2004). Except for the overall deficit of sanitary facilities, free basic water is allocated to these communities while no infrastructure is in place to divert the used water to water treatment plants for purification (Tempelhoff, 2009). Thus, it ends up in the rivers where it threatens the already fragile ecosystems. According to the General Household Survey (Stats SA, 2005), 68% of households in rural communities have access to drinking water of improved quality while only 10.2% have access to adequate sanitary facilities.

Human excreta from informal settlements ends up in containers meant for the collection of solid waste, but many (40%) do not receive regular removal of waste (Stats SA, 2005). Apart from the implications this may have on the health of members of communities coming into direct contact with this waste, contamination of nearby water sources becomes a problem. The increased volumes of water being used in formal and informal settlements alike, without the upgrading and proper maintenance of wastewater treatment plants are one of the biggest factors in the pollution of the country's freshwater resources.

For many communities living in unserviced informal settlements it is not unusual to use the riverbanks or even the river itself as a place to relieve themselves, thus it comes as no surprise that these waters contain vast amounts of faecal coliforms (Okafu *et al*., 2003). In many areas these polluted sources are used for domestic purposes by the communities, by downstream users for farming activities and even for recreational purposes such as swimming and fishing. This emphasises the need for better sanitary services and hygienic practices in informal settlements, as well as the upgrading of water treatment works and the adherence to strict quality standards for effluents to be released into rivers (Okafu *et al*., 2003). To support and maintain river ecology many treatment plants release partially purified water into the nearest freshwater source (Britz *et al*., 2007). In many cases however, these effluents do not comply with the regulations set out by the Department of Water Affairs and Forestry (Tempelhoff, 2009). The main reasons for this being poor maintenance and management of equipment and the increased volume of wastewater generated by nearby communities (Britz *et al*., 2007; Tempelhoff, 2009).

Research done by Venter *et al*. (1997) showed that effluents received from four wastewater treatment plants and runoff from a nearby informal settlement with insufficient sanitary facilities had a greater negative impact on the microbial quality of a river than the effluent the river received from an industrial area. The study also indicated that the dilution factor of the river water and the decay of the organisms was not enough to improve the microbial quality of the water to an acceptable level. According to a study by Barnes & Taylor (2004), the Stellenbosch waterworks had been releasing improperly treated effluent into the Eerste River for several years. During the summer months, this constituted as much as 80% of the flow and is probably the main reason for the degradation of the river ecology. For many farmers in the region, this river is the only source of irrigation during the dry summer months. Although they are cautious of using this tainted water, they are forced to use it if they want to be assured of a harvest as no alternative sources are available.

In many developing countries focus is placed on assuring there is enough water for years to come, and large amounts of money are invested in projects with this solitary goal (Scott *et al.*, 2004). What people tend to forget is that as much as 70% of the water used for domestic purposes is returned as wastewater (Faruqui *et al*., 2004). If this water is not disposed of in a sanitary manner, it can easily contaminate other water sources (Scott *et al.*, 2004). As the population of South Africa continues to grow the volume of wastewater generated is increasing as is the demand for fresh water and food (Scott *et al.*, 2004). Although more and more urbanised households are connected to sewers (Table 1), there is still a large part of the South African population that makes use of septic tanks and other means to get rid of wastewater, and often this raw or partially treated wastewater seeps into rivers and other surface water bodies (Scott *et al.*, 2004).

Another of the many examples is the degradation of the water quality of Lake Chivero in Harare, Zimbabwe. This water body received purified wastewater from the city and in turn supplied the two million inhabitants with fresh drinking water. The last couple of years have seen the quality of the incoming water slowly decreasing as population growth and migration led to bigger volumes of wastewater being generated. The partially treated sewage makes its way into the lake where it disrupts the natural ecology which leads to the growth of algae. Apart from the taste, smell and colour of the water being affected it does not readily form sediment – the end result being clogged filters and an increase in the pH, alkalinity and turbidity of the water which in turn makes it hard to purify. Further implications for the Zimbabwean consumer are that the cost of purification is so much higher and subsequently this leads to increases in the price of drinking water (Madungwe & Sakuringwa, 2007). Furthermore, the poor state of the country's sanitary infrastructure could be the cause of the cholera outbreak Zimbabwe is battling to overcome (Chambers, 2009). Power cuts, sewer bursts due to a lack of maintenance, the deficit of clean, piped water and a shortage of man-power all contributed to the biggest cholera outbreak to date – a staggering 89 018 infections and 4 011 deaths noted by 9 March 2009 (Chambers, 2009; Cooke & Shapiro, 2009).

As can be seen from the statistics mentioned above, all of the Southern African regions are confronted with a challenge that will increase as their population increases (Scott *et al.*, 2004; St. Louis & Hess, 2008). In countries where the rate of the treatment of wastewater and the supply of fresh water are lower than the growth of the population, the demand for fresh food products will necessitate the use of any water for agricultural purposes, even if it is untreated wastewater (Scott *et al.*, 2004). In regions where water-scarcity is a reality, farmers often make use of wastewater as it is rich in nutrients and,

most of all, a reliable source of irrigation. Even though the microbial safety of the produce is questionable, people will still eat it if it is the only food they can afford or have access to.

The South African population has since 1975 had an annual growth rate of 3.34%, with the numbers increasing from 5.17 million in 1904 to 47.4 million in 2006 (DEAT, 2006; Stats SA, 2006). Although the population has increased by eightfold, the natural resources did not and we are compelled to survive with the same amount of water. As a consequence of the HIV/AIDS epidemic, the population growth rate and the life expectancy in South Africa is now 12 years lower than in 1996. At present, more than 5.7 million South African citizens are infected with the HIV/AIDS virus (Kapp, 2009).

Table 1 Wastewater treatment and sewered areas by world region (Scott *et al.*, 2004).

Region	**Population (%) in large cities that is sewered**	**Sewered wastewater (%) that is treated to secondary level**
Africa	18	0
Asia	45	35
Latin America/ Caribbean	35	14
Oceania	15	Not reported
Northern America	96	90
Europe	92	66

Even though South Africa is seen as a net-exporter of fresh produce and the country is listed as food secure, more than 35% of families, especially in rural areas, are vulnerable to food shortages and 8.9% stated that they or their children often went hungry (Stats SA, 2005). Nationally, three out of four families have a diet lacking in sufficient energy and most of the essential nutrients (NFCS, 2000). Most impoverished communities have a diet mainly consisting of plant-based staple foods such as maize meal and the consumption of fresh produce such as fruit and vegetables is rare. As vegetables and fruit are excellent sources of many of the macro and micro nutrients, the planting of home-based gardens is being encouraged. Unfortunately many of these individuals only have access to water of poor microbial quality, and the vegetables grown to boost their health are now putting them at a greater disease risk. Amplifying this risk of infection is the fact that these individuals already have a weakened immune system due to malnutrition and the ingestion of an even a small number of pathogens may lead to infection (Britz *et al*., 2007).

According to Capra & Scicolone (2007), the model of social and economical development in Italy shows the migration of people from rural to urbanised areas. The development of urban areas goes hand in hand with the development of the tourism industry. The same phenomenon can be seen in our country and is very applicable (WESGRO, 2008). Both developing urban areas and increasing tourism activity demands greater volumes of fresh water which inevitably leads to the production of greater volumes of wastewater. This will become a huge problem. Even though excellent purification methods exist, the budget for the maintenance of the water treatment plants often do not follow the pattern as that of the water usage – especially not in developing countries (Capra & Scicolone, 2007).

AGRICULTURE IN SOUTH AFRICA (WESTERN CAPE)

The consumption of fresh and minimally processed vegetables is gradually increasing in many countries. Industry has met this increasing demand by making use of various packaging and distribution methods and intensive farming techniques (Beuchat, 1996). The agricultural industry has been under severe pressure in recent years, not only to supply the increasing population with fresh food, but to use as little fresh water as possible in doing so, as water is becoming a very scarce resource (NWRS, 2004).

South Africa is considered the continent leader when it comes to the export of fresh produce (Ndiame & Jaffee, 2005). As much as 73% of the continent's fruit intended for export to the USA originates from within the country's borders. As the second largest exporter of apples, pears, stone fruit, peaches and plums in the southern hemisphere, 60% of all the fruit cultivated in SA is exported and it comes as no surprise that we currently hold 31% of the European Union's market share for imported fruit. Of the remainder, 20% is consumed locally and 20% is further processed into juices for the retail market (WESGRO, 2006). South Africa can easily compete with leading export countries, and is currently ranked in the top 20 countries for the export of several products (Table 2). According to the National Department of Agriculture (NDA, 2007), the produce sold in the biggest annual volumes at fresh produce markets on own turf are: tomatoes (255 800 t), potatoes (895 200 t), cabbage (129 300 t), onions (283 000 t), carrots (89 400 t) and butternut (80 400 t). The cultivation of various fruits has increased significantly in the period between 1980 and 2005. These values, along with the destined use and the economical values of these products are given in Table 3.

With the favourable climatic conditions, the Western Cape produces as much as 70% of all the fruit cultivated in South Africa, contributing 25% of the sector's total gross

income and between 55 and 60% of the produce intended for export (WESGRO, 2008). More than 20% of the citrus and 12% of the country's vegetables are grown in the province, the main vegetables cultivated being potatoes, onions, carrots and cabbages (WESGRO, 2003). Apart from being the leader in the cultivation of fruit and vegetables with exports to the value of R7 billion per year, the Western Cape agricultural sector is the primary employer in this region. As much as 8 500 commercial farmers, 2 500 new development farmers and 220 000 farm workers form part of this industry, in turn supporting more than 1.5 million dependants (WESGRO, 2006).

The agricultural activities account for 2.6% of the country's annual gross domestic product (GDP) and gives formal employment to as much as 9% of the population (WESGRO, 2006). These, however, are only direct measurements of the sector's contribution and if more accurate approximations are made, it may be in the region of 20 – 30%. These estimates then include the contributions of the industry on the food supply, direct and indirect employment and foreign exchange earned through trade with other countries (Backeberg *et al.,* 1996; Nieuwoudt & Groenewald, 2003).

It has been reported through studies done in the Middle East, South America and Asia that farmers prefer to grow crops in a certain order of preference (Smith & Shaw, 2007):

1. Vegetables, as this ensures a steady income;
2. Fruit, especially for export purposes;
3. Cereal crops, these do not generate such a great income but can be grown extensively;
4. Fodder crops, used as feed for livestock or sold to others for the same purposes;
5. Herbs, flowers and spices, depending on the consumer demand.

Table 2 South African produce intended for export and the corresponding position amongst leading exporting countries (Harris *et al*., 2003; FAO, 2005).

Produce	Quantity (Mt*)	Value (US $)	Position amongst leading 20 exporting countries globally
Apple	305 190	181 020 000	9
Avocados	28 585	21 153 000	7
Carrots	1 832	1 994 000	19
Grapes	237 110	282 786 000	4
Mangoes	9 919	8 236 000	13
Oranges	736 592	270 667 000	3
Pears	138 836	79 626 000	8
Pineapples	3 774	3 325 000	20
Potatoes	30 319	9 733 000	20
Sweet Potatoes	470	267 000	20

*(Mt) =Metric tons

Table 3 Increase in production, destined uses and economical value of nationally grown fruit (NDA, 2007).

Product	1980's (t*)	2000's (t)	National consumption (%)	Export (%)	Processed (%)	Value 2000's (SAR#)
Apples	394 164	35 594	29	38	28	1 447 033 000
Apricots	-	43 741	3	8	73	83 119 000
Pears	136 208	329 165	-	46	34	775 302 000
Peaches	165 871	184 495	-	-	70	359 227 000
Plums	9 539	54 591	-	72	-	246 918 000
Strawberries	-	4 851	45	-	55	38 499 000
Guavas	-	28 278	-	-	85	32 027 000

*t = tons; #SAR = South African Rand

Table 4 Gross Geographical Product Statistics for Agriculture, forestry and fishing in 2005 (WESGRO, 2006).

	South Africa (SAR)	Western Cape (SAR)
Gross geographical product	34 411 000 000	7 453 000 000
Export of goods	15 874 000 000	7 604 000 000
Import of goods	4 755 000 000	783 000 000

[#]SAR = South African Rand

In 2002 it was estimated that there were 2 500 growers of deciduous fruit in the province (WESGRO, 2002) and that their produce accounted for 85% of the country's total deciduous fruit exports. This equals 2% of the world's apples and approximately 1% of the world's pears (WESGRO, 2002). WESGRO (2007) has observed a change in the province's agricultural sector in recent years as international and local consumer trends changed and the industry adapted itself to deliver an array of organic products and health foods. Even with the international economic environment smothering export activities, the province's exports in 2005 were valued at R37 937 million (WESGRO, 2007) and exports to countries like Vietnam, Nigeria, Malaysia and Singapore are still growing. Table 4 shows the Rand value of the gross geographical product, and import and export values of the Western Cape against that of South Africa in 2005. Export from just the Western Cape has grown to over R8 billion over the last years (WESGRO, 2006). Looking at the figures listed in Table 4 above, one can see what role agriculture in the Western Cape plays in not only the economy of the province, but also of the country. It is therefore of great importance that all necessary steps be taken to protect the Western Cape rivers - and in so doing the export licenses of farmers.

IRRIGATION AND THE USE OF IRRIGATION WATER

Backeberg *et al.* (1996) estimated that the agricultural industry was made up of 40 000 small-scale farmers, 15 000 medium-scale farmers, 120 000 farm workers and an indefinite number of seasonal workers in 1996, and for that specific year 51% of the country's fresh water was utilised in irrigation giving an agricultural output of 30%. The most widespread sources of irrigation water used by South African farmers are rivers, farm dams, large reservoirs, groundwater, municipal supplies and industrial effluents (Britz *et al*., 2007).

While the agricultural sector is well developed, the agricultural activities in rural areas of South Africa are mostly subsistence-orientated. Only 22% of the country's available land is suited for agricultural purposes, and of this percentage only 13% can be seen as land with great agricultural potential - the most important factor limiting these possibilities being the availability of water (Britz *et al.,* 2007). Between 1956 and 1986 as much as 27% of the country experienced droughts for more than half of the time and it would therefore seem that the land is more appropriate for the grazing of cattle (Cowling, 1991).

Although there are large inter-provincial differences in the suitability of land for irrigation (Table 5), the national area suitable for this purpose is estimated at around 1 498 000 ha, the main crops cultivated being wheat, sugar cane, vegetables, silage and pulses (FAO, 2005). Between 25 and 30% of South Africa's crops for both the local and export markets are cultivated on irrigated land – this accounts for some 90% of the vegetables, deciduous fruit, grapes and citrus produced (Backeberg *et al*., 2006). The use of irrigation water for the cultivation of fruit and vegetables is crucial as the economy of the country depends heavily on this industry for the generation of foreign exchange. Any negative changes in this sector could affect the country's trading status, employment and other industries.

The Food and Agricultural Organisation (FAO, 2005) reported that three key irrigation application designs are used globally. Surface irrigation, mechanised and non-mechanised sprinkler systems and localised irrigation being the chosen methods, the choice between these are influenced by factors such as soil type, the availability of water, the depth of the water table, economics and cropping rotations. South African farmers, irrespective of the rainfall region, mostly make use of permanent structures for irrigation (Backeberg *et al*., 1996) and these include sprinkler irrigation (54%), flood irrigation (33%), and micro-irrigation (12%).

Due to the absence of infrastructure where the treatment of wastewater is concerned, and the overall shortage of water in arid and semi-arid countries, the use of water of uncertain microbial quality or even reclaimed water is used for irrigation purposes (Gómez *et al.*, 2006). Normal treatment processes may remove as much as 99% of the organisms present, but still this water is not suitable for direct human exposure as it may contain potential human pathogens. Presently treatment works rely on chlorine to inactivate pathogens in waters, but several studies have reported that turbidity, suspended solids and the presence of other compounds hinder the effectiveness of this process (Gómez *et al.*, 2006). In addition to these limitations, some microorganisms may be

resistant enough to survive this type of treatment and the diverse array of organisms found in wastewaters will not be removed with equal efficacy (Barnes, personal communication, 2007). Parasite ova, cysts and even some viruses are known to be resistant to chlorination and UV irradiation (Gómez *et al.*, 2006).

Table 5 Provincial distribution of irrigated land in South Africa in 2000 (FAO, 2005).

Province	Permanent commercial irrigation (ha)	Temporary commercial irrigation (ha)	Total area equipped for irrigation (ha)
Eastern Cape	11 070	179 995	191 065
Free State	46	68 764	68 810
Gauteng	18	16 330	16 348
Kwazulu-Natal	2 747	131 974	134 722
Mpumalanga	18 494	116 977	135 475
North West	706	114 094	114 801
Northern Cape	34 759	130 181	164 940
Southern Cape	58 704	160 617	219 321
Western Cape	290 204	162 325	452 529
Total	**416 753**	**1 081 257**	**1 498 010**

As water used for irrigation may contain a high concentration of human excrement, there is an increased risk that consumers may contract a foodborne infection when ingesting these irrigated crops (Hamilton *et al*., 2006). Except for the possible pathogenic bacteria that may be contained and the health risk this entails for agricultural workers and consumers alike, wastewater may contain various chemicals in amounts that may retard the growth or even harm the crops (Smith & Shaw, 2007). Microbial guidelines of wastewater used for irrigation purposes (Table 6) as set out by the World Health Organisation (WHO, 1989) state that, if produce is to be eaten raw, no more than 1 000 faecal coliforms in 100 mL of water, and less than one helminth egg per litre of water must be present.

In a study by Okafu *et al*. (2003), it was found that the irrigation water contained significantly higher amounts of coliforms in the dry season than the wet season, and that the microbial loads present on the different vegetables echoed this. Thus, it must always be taken into consideration that seasonality has an important effect on the microbial quality

of produce. The vegetables with large surface areas exposed to the irrigation water also reflected higher counts of the indicator organisms in a sample of equal size. Therefore the type of vegetable grown should also be considered when irrigation water of uncertain microbial quality is used.

There are many reasons for the use of wastewater as a source of irrigation. Water-scarcity is probably the most important, followed by the constancy of the wastewater supply, the nutrient value and the economic dependence of the farmers with no other option than to use wastewater (Scott *et al*., 2004). Poor rainfall and the high expense to obtain groundwater are other contributing reasons (Buechler, 2004). The main reason why a negative connection is made to wastewater irrigation is the risk it holds for the health of the farm workers, the vendors that sell the products, and the consumers (Carr *et al*., 2004). No other solution to minimise the microbial risks when using wastewater exists except for proper treatment (Buechler, 2004). Although some cities can afford to treat most of the wastewater they generate, partially treated wastewater is used to water public gardens or sold to golf courses for irrigation purposes.

Table 6 Recommended microbiological quality of water used for irrigation purposes (WHO, 1989).

Category	Re-use conditions	Exposed group	Intestinal nematodes*	Faecal coliforms**	Treatment needed to achieve the required microbial quality
A	Irrigation of crops likely to be eaten uncooked, sports fields, public parks	Workers, consumers, public	≤1	≤1000	A series of stabilization ponds designed to achieve the quality indicated, or equivalent treatment
B	Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees	Workers	≤1	No standard	Retention in stabilization ponds for 8-10 days or equivalent removal of indicated organisms
C	Localised irrigation of crops in category B if exposure to workers and the public does not take place	None	NA	NA	Pre-treatment as required by the irrigation technology, but not less than primary sedimentation

*arithmetic mean no. of eggs per litre; **geometric mean no. per 100 ml.

The level to which wastewater is used in global irrigation is not currently known (Van der Hoek, 2004), but it is estimated to be as much as 20 million ha in 50 countries (Hussain *et al.*, 2001) producing as much as 40% of the global food supply (Gleick, 2000). It is clear that wastewater can be an important resource in the future. The challenge, however, is to minimise the health risks as far as possible (Table 7) and to allow the safe reuse of this very limited resource (Carr *et al*., 2004).

Drip irrigation is seen as the safest and most effective way to limit water shortages in the agricultural industry. The biggest problem with this method of irrigation is that the emitters become clogged if the water is not of good quality. A value of 50 mg per litre Total Suspended Solids (TSS) is given as the point after which the uniformity of irrigation will be affected (Capra & Scicolone, 2007). In the southern Mediterranean region irrigation uses between 50 and 85% of the total available water and this is implicated as the main factor influencing the size of the harvest. Sufficient irrigation may double a harvest! Because of the drought in the Mediterranean region, farmers are inclined to use wastewater for irrigation as no other suitable source is available. Irrigation with wastewater also has many advantages: valuable freshwater is not wasted on irrigation, the contamination of rivers is kept to a minimum, the nutrients in the wastewater are not lost but can be used in agriculture, and the cost of water purification is kept to a minimum (Capra & Scicolone, 2007).

The largest portion of freshwater in Africa is used for agricultural purposes and thus the use of greywater for irrigation may lead to municipal sources being available for other purposes. This will additionally lead to the reduction in cost. However, the problem is that systems are not in place in South Africa to assure that this type of water is of sound microbial quality. For this to be a success, all involved parties should be properly educated (Madungwe & Sakuringwa, 2007).

IRRIGATION WATER AND POTENTIAL PATHOGENS

At the turn of the century only five organisms directly linked to foodborne infections had been identified (Wadhwa *et al*., 2002). At present more than 40 such organisms are commonly associated with illnesses contracted from food or water, with symptoms ranging from a slight fever to lethal septicemia or even the Guillain-Barré Syndrome. Despite major advances made in the preventative health arena over the last century, foodborne diseases still remain a significant problem – one that is grossly underestimated as many third world countries have no food safety systems in place where these cases have to be reported (Britz *et al*., 2007).

The main sources of contamination of vegetables are from soil, water and air (Wadhwa *et al*., 2002), although the recent increase in the use of reclaimed water for irrigation has seen the rise of a new and definitely significant threat to the industry as well as consumer health.

Over the years researchers have seen that all microorganisms can be categorised into three groups depending on the relationship in which they associate with humans (Wadhwa *et al*., 2002). Some organisms can live inside the human body in complete harmony and are known as the "symbionts". These microbes will not cause infection or harm their host in any way, and in some cases may even be beneficial through the production of secondary metabolites such as Vitamin K. The second group, the "opportunists", will have no detrimental effects on their host under normal conditions, but they possess enough virulence to cause illness if the individual has a stressed immune system. The last group are the "pathogens". These organisms are virulent enough to infect even a healthy individual under normal physiological conditions (Wadhwa *et al*., 2002; Gourabathini *et al*., 2008). The more virulent the organism, the smaller the number of organisms needed to cause infection.

Table 7 Limiting values for various uses of treated wastewater in the US (USEPA, 1992).

Reuse of water	**Type**	**Purposes of the treatment (limiting values)**		
		BOD* ($mg.L^{-1}$)	SS** ($mg.L^{-1}$)	Thermo coli*** ($MPN.100mL^{-1}$)
Agricultural irrigation	Food crops	10	-	ND
	Non-food crops; food crops consumed after processing	30	30	200
Urban	Unrestricted	10	-	ND
	Restricted access irrigation	0	30	200
Recreational	Unrestricted	10	-	ND
	Restricted	30	30	200
Environmental enhancement		10	-	ND
Industrial reuse		30	30	ND
Groundwater recharge		Site specific		
Potable reuse		Drinking water requirements		

*BOD = Biological Oxygen Demand; **SS = Suspended Solids; ***Thermo coli = Thermo tolerant coliforms; ND = Not detected

As a vast number of potential pathogens can be found in just about any place on earth, it is nearly impossible to detect and quantify each type of these organisms (Dewedar & Bahgat, 1995; Savichtcheva & Okabe, 2006), and therefore "indicator" organisms are used to simplify things. An indicator is defined as an organism which shares the same habitat as the pathogens under observation. In the case of a faecal indicator, the organism should always be found in faeces, it would not be able to multiply outside of the intestinal tract, it would be more or less as resistant to environmental factors and disinfectants as the pathogens themselves, a strong correlation should exist between the presence of the pathogens and the indicator, and the organism should be relatively easy and safe to cultivate and enumerate under laboratory conditions (Savichtcheva & Okabe, 2006).

Total coliforms, faecal coliforms and *Enterococci* species are widely used as indicators of faecal contamination in water bodies (Savichtcheva & Okabe, 2006) although several intrinsic properties make them less than ideal for this purpose. These include their rapid decay rate, their ability to proliferate outside the intestinal tract, their inability to withstand disinfectants, their shortcomings in identifying the source of faecal pollution and the fact that they can be of non-faecal origin, problems with cultivation under laboratory conditions and a low association with the presence of enteric pathogens. It can be concluded that, as yet, a single organism that can be used as an indicator of faecal pollution, has not been identified (Savichtcheva & Okabe, 2006) as the survival characteristics and decay-rates of different pathogens vary under identical environmental conditions (Savichtcheva & Okabe, 2006). At this stage researchers agree that, although not ideal, faecal coliforms and *E. coli* are the best indicators of faecal pollution available.

Bacteria

These metabolically active organisms are capable of self-replication, but more often than not environmental factors impair the replication process (Toze, 2006). Except for their water or foodborne transmission, humans can also be infected with these organisms by direct contact with animals which serve as carriers.

Total Coliforms - Organisms that form part of the total coliform group are defined as being aerobic or facultative anaerobes. They are Gram and oxidase negative, catalase positive and have the ability to ferment lactose at 35ºC with the formation of acid and gas as end-products. These organisms are rod-shaped and do not possess the ability to form endospores (Schraft & Watterworth, 2005). As this group is defined by biochemical characteristics rather than taxonomic nature, it automatically includes various genera such

as *Klebsiella*, *Escherichia*, *Citrobacter* and *Serratia*. Both faecal and environmental species are included in this group, so total coliforms alone cannot be used as an indication of faecal pollution. This group can, however, be seen as an indication of the effectiveness of water treatment processes.

Faecal or thermotolerant coliforms - These organisms are a sub-group of the coliforms and possess the ability to ferment lactose at an elevated temperature (44ºC), and in so doing produce the enzyme ß-galactosidase. In many instances the number of thermotolerant coliforms in a sample are more or less similar to the number of the *E. coli* in the same sample (Schraft & Watterworth, 2005). It is, however, necessary to do further biochemical tests to distinguish between the two groups (WHO, 1993). In tests done by Schraft & Watterworth (2005), a positive identification of a faecal coliform required that the isolated organism be a Gram negative and oxidase negative rod with an additional positive outcome when subjected to the Lauryl/EC test.

Escherichia coli - *E. coli*, a member of the *Enterobacteriaceae* family, is a normal inhabitant of the intestinal tract and is at present seen as the most appropriate indicator of faecal contamination in drinking water (WHO, 1993). While many of the strains are harmless, some like *E. coli* O157:H7 are the etiological agent in many foodborne outbreaks and may result in haemorrhagic colitis, gastroenteritis and kidney failure leading to death if no medical intervention is taken (Francis *et al.,* 1999; Velazquez *et al*., 2009). This specific strain is indicated in 34% of all foodborne *E. coli* outbreaks, and increasing levels of this organism are being isolated from river systems. *Escherichia coli* is resistant to acidic environments and can easily survive at pH values between 3.3 and 4.2. This organism flourishes in an oxygen-rich environment, but Gill & Selma (2006) reported that the use of modified atmosphere packaging (MAP) had no detrimental effect on the organism. A Gram negative and oxidase negative rod could be identified as *E. coli* when it exhibits a specific (++--) pattern after being subjected to the IMViC test (Schraft & Watterworth, 2005).

Staphylococcus - The genus *Staphylococcus* includes as many as 27 species and a number of subspecies. These organisms are Gram positive, catalase positive cocci with a preference for high salt foods (Wadhwa *et al.,* 2002). *Staphylococcus aureus*, *S. intermedius* and *S. helviticus* are the enterotoxin producing members of this genus, and although only five foodborne outbreaks linked to these organisms have been reported

between 1996 and 2005 (Velazquez, 2009), they remain a significant health risk as they are opportunistic pathogens.

Intestinal Enterococci and Faecal Streptococci - These Gram positive, catalase negative cocci (Wadhwa *et al.,* 2002; Johnston & Jaykus, 2004) are homofermentative, facultative anaerobic organisms. The most well know human pathogens of this genus include *Streptococcus pyogenes* and *S. pneumoniae*, and several studies have reported the isolation of these organisms from frozen vegetables, making them a threat worth mentioning.

Spore-forming bacteria - Members of the genera *Bacillus* and *Clostridium* are Gram-positive, spore-forming rods (Wadhwa *et al.,* 2002; WHO, 2004). *Bacillus cereus* has been implicated in foodborne outbreaks linked to sprouts and this comes as no surprise as the organism is ubiquitous to plant material and soil (Harris *et al.,* 2003). Of the genus *Clostridium*, two species are of great concern where fresh produce is implicated. *Clostridium perfringens* is often found in the faeces of humans and animals and has been isolated from river water on occasion (Harris *et al.,* 2003). The neurotoxigenic *C. botulinum* is the etiological agent for a condition known as botulism (Kautter *et al.,* 1992). These anaerobic bacilli are often isolated from improperly processed canned foods, where they proliferate and produce the deadly neurotoxin, botulin. Although this foodborne infection is rare it claims many lives when outbreaks occur (Kautter *et al.,* 1992).

Salmonella - According to Flowers *et al.* (1992) the occurrence of salmonellosis in America has increased between 1970 and 1990, with averages between 740 000 to 5 300 000 incidents per year with leafy greens contributing greatly to these outbreaks (Elviss *et al*., 2009). This very resistant organism also forms part of the *Enterobacteriaceae* family and includes five pathogenic strains, namely *S. typhimurium*, *S. enteritidis*, *S. heidelberg*, *S. saint-paul* and *S. montevideo* (Francis *et al*., 1999). With optimum growth temperatures between 35℃ and 43℃ and very low oxygen demands, this organism thrives in the human body making it a very effective human pathogen and the second largest cause of diarrhoea (ECSCF, 2002).

Listeria - *L. monocytogenes,* a Gram and catalase positive organism, is ubiquitously found on all plant materials, including vegetables and fruit (Beuchat, 1996; Conter *et al*., 2009). This specific *Listeria* species has the capability to survive many environmental conditions including low oxygen concentrations, temperatures as low as 0.5℃ and high moisture levels making it ideal for growth in aquatic environments (Francis *et al*., 1999). A further

example of this oxidase negative, rod shaped organism's resistance is that Brackett (1987) found hypochlorite to be unsuccessful in the decontamination of vegetables infected with *L. monocytogenes*. Studies have shown that this organism can survive for as long as 12 years under ideal environmental conditions (Beuchat, 1996). In a study by Welshimer (1960), it was shown that the number of *L. monocytogenes* organisms present in a soil sample barely decreased during a seven week period after irrigation with sewage sludge, and that the organisms survived for up to 295 days. The long incubation period of listeriosis makes it extremely difficult to trace back to the origin or source of the contamination. This disease is very serious and can result in meningitis, still-births and natural abortions (Francis *et al*., 1999). It appears that *L. innocua* and *L. monocytogenes* are more prevalent in faeces, while *L. ivanovi* and *L. seeligeri* are commonly found in soil samples (Beuchat, 1996).

Shigella - In view of the fact that the infective dose for the genus *Shigella* varies between 10^1 and 10^4 cells per person, and infection occurs through faecally contaminated water or food (Smith & Buchanan, 1992; CDC, 2008), great care should be taken when irrigating minimally processed foods with water of questionable microbial quality. Grouped as part of the family of *Enterobacteriaceae*, these non-motile organisms are Gram and oxidase negative, catalase positive facultative anaerobic bacilli. Virulence occurs only at temperatures around 37℃ and secretion of an exotox in takes place (Smith & Buchanan, 1992).

In countries where chlorinated water is used Shigellosis, a contagious infection of the colon also known as bacillary dysentery (Van Elfen, 2001), is more often linked to a contaminated food source (Smith & Buchanan, 1992). Symptoms such as profuse diarrhoea (usually containing blood and mucus), abdominal cramps and dehydration appear within 12 to 50 hours after ingestion, and can last for up to 14 days (Smith & Buchanan, 1992). Foodborne outbreaks of the disease are usually linked to the use of raw, contaminated products in salads or foods that have not been properly cooked before consumption (Smith & Buchanan, 1992).

Where water is contaminated with faeces of animal origin, this pathogen may be present (Savichtcheva & Okabe, 2006). Brackett (1999) considers the group *Shigella* as a threat to human health in cases where fresh produce is irrigated with contaminated water and then consumed raw.

Plesiomonas - The only species in this genus, *Plesiomonas shigelloides*, is an opportunistic enteropathogen commonly associated with illness after ingestion of raw seafood (Smith & Buchanan, 1992; Koburger & Wei, 1992). Motility, facultative oxygen needs and the presence of oxidase and catalase are some characteristics of these gram-negative rod-shaped organisms (Smith & Buchanan, 1992).

After ingestion, symptoms appear within one to two days and include abdominal cramps, diarrhoea, nausea and fever. Dehydration and loss of important electrolytes may occur if patients are not diagnosed and treated rapidly (Smith & Buchanan, 1992). The organism thrives in water, whether it be fresh- or ocean waters (Smith & Buchanan, 1992), and therefore can be considered a potential pathogen occurring on irrigated produce worldwide (Brackett, 1999).

Yersinia - This rod-shaped organism is not frequently found on food, but has been isolated from fresh produce (Velazquez *et al*., 2009) and can be classified as part of the *Enterobacteriaceae* family (WHO, 2004). It is Gram negative, oxidase negative and a facultative anaerobe and is able to ferment substrates to obtain the necessary nutrients (Schiemann & Wauters, 1992). Three species of *Yersinia* are known to be human pathogens: *Y. pestis*, which is the organism responsible for plague, *Y. pseudotuberculosis*, which is not normally linked to foodborne diseases, and *Y. enterocolitica* which is more often linked to foodborne outbreaks than the other two species. Although the organism is not usually associated with food such as fresh fruit and vegetables, evidence exists that it is present in the faeces of animals (Hurst *et al*., 2002). If animals are allowed to graze near rivers or other surface-water bodies, their excreta can easily end up in water that may be destined for the irrigation of minimally processed foods. Seeing that the species are psychrotrophic, it can survive and multiply at refrigerator temperatures (4ºC) without difficulty. This is important if you consider that fresh fruit and vegetables are usually kept at low temperatures to prolong their shelf-life. Its biochemical activity is visibly higher at 25℃ than 35℃ in that it gives a positive result f or the Voges-Proskauer test and exhibits motility only at 25℃ (Schiemann & Wauters, 1992). Not many outbreaks of Yersiniosis, the disease associated with a *Yersinia* infection, have been reported (Schiemann & Wauters, 1992) and in the event of a foodborne outbreak it was not linked to either fresh fruit and vegetables or irrigation water.

Vibrio - The genus *Vibrio* includes at least three species that are known as human pathogens: *V. cholera* which is the etiological agent in cholera, *V. parahaemolyticus* which

is often found in seafood and seawater, and *V. vulnificus* that causes septicemia (Kaysner *et al*., 1992). These organisms can be described as Gram negative, curved, motile rods that do not form endospores. Most *Vibrio* species can ferment glucose without the formation of gas and are oxidase and catalase positive. *Vibrio parahaemolyticus* infections are usually associated with raw fish and seafood, and pose a serious threat to the health of especially Japanese citizens, seeing as they eat most of their fish raw (Kaysner *et al*., 1992). Breaks in the cold chain or unsatisfactory hygiene practices are given as other possible reasons for foodborne outbreaks (Kaysner *et al*., 1992). Most cholera patients contract the disease via the faecal-oral route through ingestion of contaminated water, or eating minimally processed or raw vegetables that were either irrigated with contaminated water, or fertilised using faecally contaminated materials or faeces. Foodborne outbreaks of the disease are also associated with raw or undercooked seafood. Symptoms appear within three days after ingestion of the organism, and include nausea, headaches and fever. Serious dehydration and loss of important electrolytes is the result of acute diarrhoea and may claim the life of a patient if they are not diagnosed and treated in time (Van Elfen, 2001). Vast amounts of the organism are isolated from the excreta of infected individuals (Kaysner *et al*., 1992) and animals (Hurst *et al*., 2002). If these excreta were to contaminate irrigation water, consumers are at great risk of contracting the disease (Brackett, 1999).

Protozoa

Protozoa are unicellular parasites which survive in a latent state called cysts or oocysts outside of a host organism (Toze, 2006). These parasites are commonly found in water, and *Cryptosporidium* species have been found globally, but seem to be more persistent in tropical regions.

Cryptosporidium - Classified as protozoa, and a serious human pathogen, this organism is often isolated from surface waters (Fayer *et al.*, 1992, WHO, 2004). They occur as oocysts, and Fayer *et al*. (1992) reported that 72% of surface water samples taken in the United States of America tested positive for these oocysts. Cryptosporidiosis is an infection of the gut, caused by *C. parvum*. After an incubation period of 12 days, the patient experiences intense stomach cramps and watery diarrhoea, while fever and vomiting are less frequently seen (Anon., 2003). Patients with a fully functional immune system will recover within two weeks, but for individuals infected with HIV this disease may be life threatening (Anon., 2003). The infection occurs through the intake of infected

drinking water or food and, because this organism is largely resistant to chlorine and antibiotics, it is extremely difficult to treat (Anon., 2003).

Helminths

Nematodes and tapeworms are ubiquitous parasites that spread via the faecal-oral route (Toze, 2006; WHO, 2004). Some of these organisms spend a part of their life-cycle in another host before they are ready to infect humans (WHO, 2004). Amongst those commonly found in wastewater *Ascaris lumbricoides*, *Ancyclostoma duodenale* and *Trichuris trichiura* do not require an intermediate host and are therefore the main causes of human helminth infections. One of the principal causes of helminth infections is the use of untreated or partially treated sewage for irrigation.

Viruses

Of all the pathogens found in water, enteric viruses are the smallest. These organisms cannot survive outside of a host organism, where they mutate the RNA of the host cell to produce copies of their own DNA. In water, however, viruses are present as inactive particles. The number of virus particles found in wastewater depends on the health of the population generating this wastewater (Gilboa & Friedler, 2008) and comprise a definite health risk because of their very low infectious dose. Infected patients secrete large numbers of viruses through defecation, and therefore the principal mode of transmission is via the faecal-oral route by means of water or food coming into contact with this faecal matter (Haramoto *et al*., 2006). More often than not, partially treated sewage contains varying numbers of the Hepatitis A virus, Noroviruses and Enteroviruses. According to Green *et al.* (2001), human noroviruses are responsible for most cases of non-bacterial gastroenteritis globally and it is estimated that more than 90% of viral gastroenteritis infections can be linked to this group of organisms. The current bacterial indicators are ineffective as viral indicators and therefore it is necessary to find suitable organisms for this purpose (Savichtcheva & Okabe, 2006).

<u>*Noroviruses*</u> – Of the four genera in the family *Calciviridae*, only two are human pathogens: *Norovirus* (NoV's) and *Sapovirus* (Barnes & Taylor, 2004). NoV's have been found to be the leading cause of non-bacterial gastroenteritis amongst all ages, especially in developing countries. After an incubation period of 24 to 48 hours, patients experience symptoms such as vomiting, non-bloody diarrhoea, abdominal cramps and fever. This may last for three to four days. Fatalities linked to NoV outbreaks have been reported (Thornton *et al*., 2004), with the highest rate of infection found in the winter months. As

the virus is shed by means of stool and vomit for up to 10 days, the contamination of surface waters is a strong possibility – especially in regions where sanitary facilities are absent or not in working order. These viruses are resistant to low pH and even heat treatments. They remain infectious even after a 30 min treatment of 0.5 to 1 mg of free chlorine per litre (Koopmans & Duizer, 2004). This could be a potential problem as a range of between 0.2 to 0.5 mg/L free chlorine is considered as the level at which drinking water is safe (WESGRO, 2008)

Hepatitis A virus – Outbreaks associated with the Hepatitis A virus (HAV) are reported to be more prevalent in areas with a dense human population such as prisons, schools and informal settlements (Koopmans & Duizer, 2004). This is to be expected as the virus is transmitted from person-to-person or spread via the faecal-oral route. After an incubation period of between 15 and 45 days, patients may experience an array of symptoms including nausea, vomiting, fever, malaise and abdominal cramps. If the illness is not identified and treated it may lead to hepatic failure, especially in non-previously exposed adults. The virus is known to survive in water for extended periods. Croci *et al*. (2002) reported the survival on lettuce and carrots to be nine and four days respectively. Resistance to extreme temperatures (60° to -20℃) a nd pH values as low as three means that the organism is quite tough, but it may be destroyed by solutions containing 10 to 15 mg of residual chlorine.

Great emphasis is placed on the education of consumers to wash all fruit and vegetables before ingestion, but studies have shown that the use of chlorine solutions have no significant effect on the microbial loads present on the products (Wadhwa *et al*., 2002).

INFECTIVE DOSE AND HEALTH IMPACTS

The possibility exists for nearly any type of fresh produce to become contaminated with pathogens, but the fact that a specific pathogen has been isolated from produce does not imply that it will cause disease in an individual (Brackett, 1999). The risk to which an individual is exposed to by ingesting food irrigated with water of poor microbial quality is a very complex aspect to assess (Wadhwa *et al*., 2002). If large quantities of *E. coli* are present in water, one cannot come to a foregone conclusion that an individual will get sick after ingestion. Several factors play a role in the number of organisms an individual has to consume before getting sick. The factors influencing the infective dose of a specific organism in a specific individual can be divided into three main groups (ICD, 2000; WHO,

2004). These include the following: host mediated factors, organism mediated factors and food or carrier mediated factors

- Host mediated factors
    - Age of the individual – young children and the elderly are more susceptible to foodborne infections;
    - Immune status – a host may acquire lasting or short term immunity against a number of organisms after exposure to them. This, however, is not possible for all pathogens;
    - Gastric acidity – gastric acidity fluctuates throughout the day and varies between individuals. As pathogens are sensitive to low pH, a host with a high amount of stomach acid will be less prone to contract a foodborne infection;
    - Immuno-competence – Individuals with a compromised immune system, like those infected with HIV/AIDS, have a greater chance of contracting a foodborne illness;
    - Microbial population of the gut – Native organisms present in the digestive tract may protect the individual against the onslaught of pathogenic microorganisms;
    - Pregnancy – it is a well known fact that the chances of contracting a foodborne infection are greater during the nine months of pregnancy.
- Organism mediated factors
    - Vegetative cells or endospores – organisms are more likely to survive the harsh conditions in the human stomach if they are in their spore-form. Once in a favourable environment, these spores will germinate and proliferate causing illness;
    - Virulence – the more virulent the strain, the smaller the amount of organisms needed to cause infection.
- Food and carrier mediated factors
    - Fat content – the high fat content of some foods may form a barrier around the organism, protecting it against the acid in the stomach and thus increasing chances of infection;
    - Acidity – Acid foods are less likely to be the carriers of foodborne pathogens as the low pH of these products will have a detrimental effect on the organism even before ingestion.

Furthermore, the risk of contracting a disease from produce irrigated with contaminated water may be influenced by factors such as the level of contamination in the

water, the persistence of the pathogens in the water, soil and on the product, and the route through which an individual is exposed to the organism (Steele & Odumeru, 2004).

In some African countries a large part of the population is infected with AIDS. According to UNAIDS (2004), of the adult populations of Botswana, Lesotho, Swaziland and Zimbabwe as many as 37%, 28%, 38% and 24%, respectively, are infected. These individuals are even more susceptible to foodborne infections and are therefore at a greater health risk when consuming crops irrigated with water of poor microbial quality.

The estimated infective dose of some pathogens frequently indicated in foodborne outbreaks is given in Table 8. These may, however, not be very accurate seeing as researchers feed the organisms to healthy volunteers in milk or sodium bicarbonate solutions. The buffer capacity of these solutions can protect the organisms against the acid in the stomach, causing an infection at a much lower dose (Kothary & Babu, 2001). However, the results do give the indication of the level that could be dangerous for healthy individuals.

Exposure to water aerosols containing high levels of human pathogens may put the health of farm workers and nearby residents in grave danger (Carducci *et al.,* 2008). Many factors influence the spread of aerosols in the atmosphere and include temperature, wind speed and relative humidity. The use of overhead sprinkler irrigation further increases the chances of infection by viral or bacterial pathogens. Studies have shown (Carducci *et al.,* 2008) that workers at various wastewater treatment plants all suffered similar symptoms such as malaise, acute rhinitis, fever and weakness. This illness was found to be of viral origin and was subsequently called Sewage Workers Syndrome.

In a study involving various types of vegetables all irrigated with faecally contaminated water from the same source, Okafu and co-workers (2003) found that all the vegetables had a viable count of 100 000 cfu.mL^{-1} exceeding the regulations of 2000 cfu.100ml^{-1} (WHO, 1975). Thirty-nine enteropathogenic *E. coli* strains were isolated and 15 of these were confirmed to be toxigenic by means of an ileal loop test. In this instance there was a definite health risk to the handlers and consumers of the vegetables. It was also found that, although all the vegetables were irrigated from the same source, varieties such as lettuce had significantly higher faecal coliform counts illustrating the importance the type of crop has on the risk of infection.

Even though microbiological standards for wastewater reuse have been drawn up by the WHO (1989) (as previously given in Table 6), many researchers are doubtful if these are strict enough to prevent the outbreak of foodborne diseases. At this stage no agreement has been reached on what these regulations should look like. One thing to

keep in mind though, is that regulations implemented in the First World with great success, may not be practical in impoverished developing countries (Capra & Scicolone, 2007).

Table 8 The estimated infective dose of some well-known human pathogens (Wadhwa *et al*., 2002; ICD, 2000; Guévremont *et al*., 2006).

Organism	Estimated number of organisms needed for infection to occur
Enteropathogenic E. coli	10^6
Enteroinvasive E. coli	10 – 100
Enterotoxigenic E. coli	10^6 - 10^8
Enteroheamorrhagic E. coli	10 – 100
S. typhi	10 – 100
Yersinia	10^9
Campylobacter	50 – 500
V. cholerae	10^6 - 10^{10}
Norovirus (NoV)	10 – 100
Hepatitis A virus (HAV)	10 – 100

Safety measures should be used whenever reclaimed wastewater is used for irrigation purposes (Mancini *et al*., 2007). These include crop restrictions, the control of human exposure and others as set out in Table 9.

An increase in the occurrence of foodborne illnesses in North America recently, focussed the attention on possible reasons for this worldwide phenomenon. These include:

- Microbes have become more resistant to disinfectants and antibiotics (WHO, 2004);
- The methods used for the detection of these organisms has improved over recent years;
- Changes in consumer lifestyle;
- The importation of various products from abroad;
- Feeding stations for large animals have increased in size;
- The growing number of immunocompromised individuals present in our societies (Wadhwa *et al*., 2002); and
- The demand for products free of preservatives and with an extended shelf-life (WHO, 2004).

Table 9 Control methods and health risks involved in wastewater re-use (WHO, 1989).

Control measures	Wastewater or excreta	Field or pond	Crop	Desirable sanitary barrier	Worker	Consumer
	Level of contamination				Level of risk	
No protective measures	High	High	High		High	High
Crop restriction	High	High	High		High	Safe
Application measures	High	High	Safe		Safe	Safe
Human exposure control	High	High	High		Low	Low
Partial treatment in ponds	Low	Low	Low		Safe	Low
Partial treatment by conventional methods	Low	Low	Low		Low	Low
Partial treatment in ponds, plus crop restriction	Low	Low	Low		Safe	Safe
Partial treatment by conventional methods, plus crop restriction	Low	Low	Low		Low	Safe
Partial treatment, plus human exposure control	Low	Low	Low		Safe	Low
Crop restriction, plus human exposure control	High	High	High		Low	Safe
Full treatment	Safe	Safe	Safe		Safe	Safe

FOODBORNE INFECTIONS

Yuk *et al*. (2006) reported that there has been a marked increase in foodborne outbreaks over the last couple of years (WHO, 2004; Velazquez *et al*., 2009; Greig & Ravel, 2009; Allende *et al*., 2009). According to Stine *et al.* (2005), one of the causative factors could be that consumers eat more minimally processed or raw produce now than they did several years ago. This increases the chances of consumers contracting a foodborne disease from contaminated fresh produce. Quite ironically, the consumers striving for a healthy lifestyle are now the ones at greater risk of contracting a disease. Could there be a link between the use of wastewater for irrigation and the increase in foodborne infections, or are there other reasons? Seeing that there is a decrease in numbers of producers, foods are held for longer periods of time and often travel greater distances before reaching the consumer, thus increasing the chances for contamination and the proliferation of organisms (Jay, 1997). Importing from foreign countries also increases the risk substantially, as the microbial quality of the produce cannot be monitored as closely as it would be if it were grown on own turf.

In recent years, methods of food processing have changed to accommodate consumer preferences (Wadhwa *et al*., 2002; WHO, 2002). The convenience-food industry is flourishing and imports of fresh produce to Western countries are on the rise. With this phenomenon we have seen a worldwide increase in the outbreaks of foodborne infections. Researchers have listed a number of probable reasons that could have contributed to these incidents. These include:

- Changing agricultural practices – the growing population and the coupled pressure to supply more food has led to the implementation of intensive farming practices and, with that, an increased transmission of disease (St. Louis & Hess, 2008);
- Vertical integration of food processing – wholesalers are buying larger volumes of products from farmers but often do not have the facilities to store these products under the correct conditions making it easier for organisms to proliferate;
- Personal consumption changes – consumers are willing to sample exotic foods, some of them are even consumed raw;
- Worldwide food distribution – products are imported and exported all over the world, many of them from countries where the food laws are not as strict as the country it is destined for;
- Nutrition – malnutrition in poverty stricken countries. Malnutrition drastically increases the chances of contracting a foodborne disease (Chambers, 2009); and
- Food preservatives – the trend in Western countries is to use less or even no preservatives in food and this increases the chances of microorganisms to proliferate in products (Wadhwa *et al*., 2002; WHO, 2004).

According to figures published by the World Health Organisation (WHO, 2004), diarrhoeal diseases are the cause of more than 1.8 million deaths per year, 90% of the deceased being under the age of five. Of these cases, 88% are caused by unsafe water supplies or an overall lack of sanitation and hygiene. If the quality of water supplies could be improved, it is estimated that between six and 25% of the fatalities could be prevented, and the improvement of sanitation in rural areas could reduce deaths by 32%. Intestinal helminth infections, such as Ascariasis, Trichuriasis and Hookworm disease globally cause more than 9 400 deaths per year (WHO, 2004). If the quality of water supplies and sanitation facilities are improved, deaths may be reduced by as much as 29% for ascariasis infection and 4% for hookworm disease. It was estimated in the 1980's that, each year more than five million deaths and one billion cases of gastroenteritis occur in children under the age of five in Africa, Asia and Latin America alone (Wadhwa *et al*., 2002). A further 1% to 5% of these cases result in autoimmune diseases such as Guillain-

Barré Syndrome, or atherosclerosis and malabsorption. In Japan in 2004, the human noroviruses accounted for 44.5% of the cases of foodborne infections (Haramoto *et al*., 2006).

According to standards set by the World Health Organisation (1989), crops that are to be eaten raw should not be irrigated with effluent that exceeds a level of 1000 coliforms per 100 mL of water in 80% of the samples taken. In most cases the contamination of vegetables with human pathogens can be linked to the use of irrigation water tainted with untreated or partially treated sewage effluent (Okafu *et al*., 2003) and consumption of such vegetables has led to cases of foodborne infection.

Several foodborne outbreaks linked to cantaloupe, tomatoes, lettuce and alfalfa sprouts have occurred in the past (Tables 10 and 11). Even though the source of contamination could not always be identified, several were linked to the use of contaminated water applied during agricultural activities (Brackett, 1999; Elizaquível & Aznar, 2008).

Table 10 Pathogenic bacteria indicated in previous foodborne outbreaks linked to fresh produce (Beuchat, 1996; Elizaquível & Aznar, 2008; Velázquez *et al*., 2008).

Pathogen	**Produce**
Shigella spp.	lettuce, green onions
Salmonella spp.	sliced tomatoes, sprouts, sliced watermelon, sliced cantaloupe, unpasteurised orange juice
E. coli O157:H7	unpasteurised apple cider/juice, lettuce varieties, alfalfa sprouts
Enterotoxigenic *E. coli* (ETEC)	carrots
L. monocytogenes	cabbage
B. cereus	sprouts

Between 1995 and 1999, contaminated sprouts were indicated in at least 11 foodborne outbreaks in America, *E. coli* O157:H7 and *Salmonella* being the causative organisms in most of these cases (FDA, 1999). Sprouts are especially susceptible (Table 12) to contamination as the same environmental conditions needed to sprout the seeds are ideal conditions for the growth of bacterial pathogens. Though the seeds are disinfected before sprouting, contamination with even one bacterial cell may lead to the

proliferation of vast numbers of potential human pathogens. In the USA several outbreaks of *E. coli* O157:H7 and *Salmonella* have been linked to the consumption of contaminated sprouts (NACMCF, 1999). In July 1999, the United States Food and Drug Administration (FDA) issued a warning to consumers not to eat raw sprouts as there had been cases of foodborne infection and sprouts were under suspicion for causing the outbreak (Anon., 1999). In 2000, an enterohaemorrhagic *E. coli* outbreak linked to water occurred in Walkerton, Ontario (Schraft & Watterworth, 2005). This led to an increased frequency of microbial testing and more stringent regulations where surface and drinking water sources was concerned.

In the 33 years between 1933 and 1966, 731 cases of Listeriosis was reported and researchers suggested a possible explanation for these infections could be the consumption of raw vegetables contaminated with *L. monocytogenes* (Beuchat, 1996). This hypothesis has been proven correct (Table 13) in several instances over the years, including a Listeriosis outbreak affecting 23 individuals in eight hospitals around Boston during 1979. In 1981, a Listeriosis outbreak occurred in Canada (Schlech *et al*., 1983). Seven adults and 34 foetuses were affected by this outbreak that could be traced back to contaminated pre-packaged coleslaw.

In many impoverished countries such as Nigeria no systems are in place where the outbreak of foodborne infections can be reported. This is to some extent also true for South Africa (Britz *et al*., 2007). For this reason no information on the extent of foodborne infections and the source of these infections exist for most African countries (Okafu *et al*., 2003). There is thus an urgent need to educate consumers on the importance of washing or disinfecting vegetables before consumption – especially if they are to be eaten raw or without further processing (Okafu *et al*., 2003).

Table 11 Produce from which bacterial pathogens have been isolated (Beuchat, 1996; NACMCF, 1999; Anon., 1999; Elviss *et al*., 2009).

Product	Pathogen	Product	Pathogen
alfalfa sprouts	*Aeromonas, E. coli* O157:H7	fennel	*Salmonella*
artichoke	*Salmonella*	green onion	*Shigella*
asparagus	*Aeromonas*	lettuce	*Salmonella, Staphylococcus, Aeromonas, Shigella, E. coli* O157:H7
basil	*Salmonella*	mungbean sprouts	*Salmonella*
bean sprouts	*L. monocytogenes, Salmonella*	mushrooms	*Campylobacter jejuni*
beet leaves	*Salmonella*	mustard cress	*Salmonella*
broccoli	*Aeromonas*	mustard sprouts	*B. cereus*
cabbage	*E. coli* O157:H7, *L. monocytogenes, V. cholera, Salmonella*	parsley	*Shigella, Staphylococcus, Salmonella*
cantaloupe	*Salmonella*	potatoes	*L. monocytogenes*
carrots	*Staphylococcus*	radish	*Staphylococcus, L. monocytogenes*
cauliflower	*Aeromonas, Salmonella*	salad greens	*Salmonella, S. aureus*
celery	*Aeromonas, E. coli* O157:H7	salad vegetables	*Shigella, S. aureus, L. monocytogenes, Yersinia enterocolitica, Salmonella*
chilli	*Salmonella*	soybean sprouts	*B. cereus*
coriander	*E. coli* O157:H7; *Salmonella*	spinach	*Aeromonas, Salmonella, E. coli* O157:H7
cress sprouts	*E. coli* O157:H7	sweet basil	*Salmonella*
cucumber	*L. monocytogenes*	tomato	*L. monocytogenes, Salmonella*
egg plant	*Salmonella*	watermelon	*Salmonella*

Table 12 Number of foodborne disease outbreaks, cases and deaths in the United States between 1993 and 1997 (Wadhwa *et al*., 2002; MMWR, 2007).

Etiology	No. of outbreaks	No. of cases	No. of deaths
Bacterial			
Bacillus cereus	14	691	0
Brucella	1	19	0
Campylobacter	25	539	1
C. botulinum	13	56	1
C. perfringens	57	2 772	0
E. coli	84	3 260	8
L. monocytogenes	3	100	2
Salmonella	357	32 610	13
Shigella	43	1 555	0
S. aureus	42	1 143	1
Streptococcus, group A	1	122	0
Streptococcus, other	1	6	0
V. cholerae	1	2	0
V. parahaemolyticus	5	40	0
Y. enterocolitica	2	27	1
Other bacterial	6	609	1
Parasitic			
Giardia lamblia	4	45	0
Trichinella spiralis	2	19	0
Cryptosporidium	-	-	-
Cyclospora	-	-	-
Other parasitic	13	2 261	0
Viral			
Hepatitis A	23	729	0
Norwalk	9	1 233	0
Other viral	24	2 104	0

Table 13 Prevalence of *L. monocytogenes* on raw vegetables (Beuchat, 1996).

Vegetable	Country	Prevalence in samples (%)
Bean sprouts	Malaysia	85.7
Cabbage	Canada	2.2
	USA	1.1
Cucumber	Malaysia	80.0
	Pakistan	6.7
	USA	2.2
Leafy vegetables	Malaysia	22.7
Potatoes	USA	27.1
	USA	21.2
Pre-packaged salads	Northern Ireland	14.3
	UK	13.3
Radish	USA	36.8
	USA	14.4
Salad vegetables	Germany	2.3
	Northern Ireland	10.6
	The Netherlands	44.0
Tomato	Pakistan	13.3
Vegetables	Italy	6.9
	Spain	7.8
	Taiwan	12.2
	UK	6.2

IMPACT OF FOODBORNE INFECTIONS ON SOUTH AFRICAN AGRICULTURE

In 2005, the European Union warned farmers in the Western Cape that they would no longer accept their produce for export if they continued to use the heavily contaminated Berg River as source of irrigation (Anon., 2005). This could have dire effects on the economy seeing that fruit and wine production are one of the biggest industries in the province, and most of this is intended for export. Even though several foodborne outbreaks have been linked to imported produce, no evidence exists that consumers are at greater risk of contracting a foodborne illness from imported produce as opposed to locally grown products (Brackett, 1999). The farming practices on specific farms play a greater role in the quality and hygiene of the specific produce.

An illustration of how devastating an foodborne outbreak can be on the economy of a sector can be seen in the *E. coli* O157:H7 outbreak in the USA (Calvin, 2006). Leafy greens, head lettuce, leaf lettuce, romaine and spinach were considered to be the top five market vegetables consumed in the USA during 2005 and had a combined value of US$ 2 140 million. After the announcement of an *E. coli* O157:H7 outbreak by the FDA in September 2006, the price and sales of spinach dropped drastically.

A decrease in the quality of food products grown will have a negative effect on the trading status and economy of the agricultural sector of any country, and can even lead to farmers losing their export licences. Without the much needed income, farmers will not be able to continue production and many individuals will lose their sole source of income (Britz *et al*., 2007). In Table 14 the gross value in South African Rand (ZAR) of some of South Africa's major produce is given along with foodborne outbreaks that have been associated with these products where irrigation water of doubtful microbial quality was used. The impact such an outbreak would have on the South African economy is clearly visible and paints an ominous picture.

According to an estimation made by the United Nations, the disposal of untreated sewage into freshwater sources is costly for many reasons, including;

- An increased rate of illness and mortality. The Global Burden of Human Disease caused by faecal pollution of coastal waters is approximated to be four million lost 'man-years' per annum, which accounts for an economic loss of US$ 16 billion. It is possible that the economic loss may be even more in the case of the pollution of fresh waters;
- Loss of earnings and added medical costs;
- Increased costs to purify drinking water and water for industrial use;
- Loss of income from the aquaculture and fishing industries;
- Costs related to the loss of biodiversity;
- A decrease in income from the tourism industry; and
- A decrease in real estate value in areas surrounding surface water bodies (Rose, 2007).

Internationally, 1 000 faecal coliforms per 100 mL of water is seen as the level above which contaminated irrigation water poses a serious risk for the spread of disease (WHO, 1989). According to South African standards, this level is only at 4 000 *E. coli* per 100 mL of irrigation water (DWAF, 2008). As can be seen, the South African regulations are less strict than those of the countries we intend to export our products to.

Table 14 Gross value of South African produce during 2005 and 2006, and pathogens indicated in foodborne outbreaks linked to irrigation water (Britz *et al.,* 2007).

Product	Value in SAR (2005/2006)	Pathogens implicated in outbreaks associated with irrigation water	Country where outbreak occurred
Potatoes	3 022 726 000	-	-
Cauliflower	24 151 000	-	-
Pumpkin	167 357 000	-	-
Beetroot	61 585 000	-	-
Lettuce	63 484 000	*E. coli, Shigella, L. monocytogenes*	USA, Norway
Carrots	168 799 000	*E. coli*	USA
Green beans	90 434 000	-	-
Green peas	36 992 000	-	-
Cabbage	94 452 000	*C. botulinum*	USA
Gem squash	36 075 000	-	-
Sweet potatoes	60 620 000	-	-
Tomatoes	841 272 000	*E. coli, Salmonella*	USA
Onions	470 278 000	*E. coli, Salmonella*	USA
Strawberries	44 917 000	-	-
Apples	1 206 996 000	-	-
Avocados	226 500 000	-	-
Grapes	1 492 197 000	-	-
Oranges	1 486 157 000	-	-
Mangoes	150 902 000	*Salmonella*	-
Naartjies	406 598 000	-	-
Pears	738 068 000	-	-
Peaches	340 272 000	-	-
Bananas	840 619 000	-	-
Grapefruit	262 402 000	-	-
Pineapples	156 063 000	-	-
Musk-melons	57 775 000	-	-
Lemons	205 205 000	-	-
Watermelons	72 050 000	-	-

DISCUSSION

The increasing rate of urbanisation and growth of the South African population has fuelled the development of widespread informal settlements. As a means to minimise their suffering, authorities have made water available to these impoverished communities who, until now, had to make use of nearby rivers for all their domestic needs. As most of these settlements are not equipped with the necessary sanitary infrastructure, the vast volumes of wastewater now generated are not purified at water treatment plants, but find their way to the nearest surface water source. This water is used for domestic purposes such as cooking, cleaning and bathing, and is often contaminated with human excreta and household detergents.

Many water treatment plants cannot keep up with the increased volume of wastewater generated by expanding urban communities. More and more houses are built and connected to sewers leading all wastewater to the nearest treatment facility, but the capacity of these facilities are rarely, if ever, increased accordingly. Apart from this, the infrastructure of many such plants is poorly maintained and, together with the lack of skilled personnel, often leads to malfunctioning of equipment and accidental leakage of partially treated sewage into nearby surface waters. Water containing high levels of partially treated human excreta is a threat to the health of any individual coming into contact with it, whether it is for domestic, recreational or agricultural purposes.

As it is one of the country's largest industries, the South African economy relies heavily on the agricultural sector, especially for foreign revenue generated by exports. Water is the limiting resource for SA's agricultural activities and irrigation is therefore necessary to assure farmers of a good harvest. With surface waters becoming increasingly contaminated with human excreta, farmers do not know which way to turn. If this tainted water is used for irrigation, their produce will not be accepted for export, but with the lack of an alternative source of irrigation they are likely to have no harvest at all.

The deteriorating microbiological quality of minimally processed foods is not only a problem for farmers wanting to export their goods. Locally, malnourished communities are encouraged to grow their own vegetables as these goods are an excellent source of various macro and micro nutrients. Unfortunately, these communities are often the ones making use of nearby streams for all their domestic needs and, being malnourished, would be at an even greater risk of contracting a water or foodborne infection.

With the use of contaminated irrigation water listed as one of the main reasons for the global increase of foodborne outbreaks, local authorities need to implement stricter regulations where the microbial quality of efficiently treated wastewater is concerned. A

drastic attempt should also be made to improve the sanitary facilities of informal settlements. In doing so, the burden of disease in these communities will be decreased while the faecal contamination of surface waters will be reduced.

As Dr Lee Jong-Wook, Director-General of the WHO said: *"Water and Sanitation is one of the primary drivers of public health. I often refer to it as 'Health 101', which means that once we can secure access to clean water and to adequate sanitation facilities for all people, irrespective of the difference in their living conditions, a huge battle against all kinds of diseases will be won."*

REFERENCES

Allende, A., McEvoy, J., Tao, Y. & Luo, Y. (2009). Antimicrobial effect of acidified sodium chlorite, sodium chlorite, sodium hypochlorite, and citric acid on *Escherichia coli* O157:H7 and natural microflora of fresh-cut cilantro. *Food Control*, **20**, 230–234.

Anonymous (1999). Consumers advised of risks associated with raw sprouts. [WWW document]. URL http://vm.cfsan.fda.gov/~lrd/hhssprts.html. 3 October 2008.

Anonymous (2003). Management of water-related microbial diseases. *Volume 1: What is the problem? Diseases characteristics*. 1st edition. The Department of Water Affairs and Forestry: South Africa.

Anonymous (2005). Berg River endangering health of economy. Cape Times, August 2, 2005.

Backeberg, G.R. (1996). Presidential Address: Constitutional change and natural resource policy reform in South Africa. *Agrekon*, **35** (4), 160-169.

Backeberg, G.R., Bembridge, T.J., Bennie, A.T.P., Groenewald, J.A., HAmmes. P.S., Pullen, R.A. & Thompson H. (1996). *Policy Proposal for Irrigated Agriculture in South Africa – Discussion Paper.* WRC Report KV96/96. Water Research Commission, Pretoria.

Barnes, J.M. (2003). *The Impact of Water Pollution from Formal and Informal Urban Developments along the Plankenbrug River on Water Quality and Health Risk*. PhD, University of Stellenbosch, South Africa.

Barnes, J.M. (2007). University of Stellenbosch, Stellenbosch, South Africa. Unpublished data.

Barnes, J.M. & Taylor, M.B. (2004). Health risk assessment in connection with the use of microbiologically contaminated source waters for irrigation. *March 2004 WRC Report No 1226/1/04*, ISBN 1-7005-010-8.

Beuchat, L. R. (1996). Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection*, **59**, 204–216.

Brackett, R.E. (1987). Antimicrobial effect of chlorine on *Listeria monocytogenes*. *Journal of Food Protection*, **50**, 999-1003.

Brackett, R.E. (1999). Incidence, contributing factors, and control of bacterial pathogens in produce. *Postharvest Biology and Technology*, **15**, 305-311.

Britz, T.J., Barnes, J.M., Buys, E.M., Ijabadeniyi, O.A., Minnaar, M., Potgieter, N., Sigge, G.O., Ackermann, A., Lötter, M., Taylor, M.B., van Zyl, W., Venter, I. & Netshikweta, R. (2007). Quantitative investigation into the link between irrigation water quality and food safety. *Water Research Commission Project Report K5/1773//4*, Pretoria, South Africa.

Buechler, S.J. (2004). A Sustainable livelihoods approach for action research on wastewater use in agriculture. In: *Wastewater Use in Irrigated Agriculture, Confronting the Livelihood and Environmental Realities* (edited by C.A. Scott, N.I. Faruqui & L. Raschid-Sally). Pp. 25-40. Oxfordshire: CABI Publishing.

Calvin, L. (2006). The economics of a produce outbreak: Spinach and *E. coli* O157:H7. IAFP Rapid Response Symposium. Arlington, Virginia, USA. 6 October 2006.

Campbell, C., Nair, Y., Maimane, S. & Sibiya, Z. (2008). Supporting people with AIDS and their carers in rural South Africa: Possibilities and challenges. *Health & Place*, **14**, 507–518.

Capra, A. & Scicolone, B. (2007). Recycling of poor quality urban wastewater by drip irrigation systems. *Journal of Cleaner Production*, **15**, 1529-1534.

Carducci, A., Tozzi, E., Rubulotta, E., Casini, B., Cantiani, L., Rovini, E., Muscillo, M. & Pacini, R. (2008). Assessing airborne biological hazard from urban wastewater treatment. *Water Research*, **34** (4), 1173-1178.

Carr, R.M., Blumenthal, U.J. & Mara, D.D. (2004). Health guidelines for the use of wastewater in agriculture: Developing realistic guidelines. In: *Wastewater Use in Irrigated Agriculture, Confronting the livelihood and environmental realities* (edited by C.A. Scott, N.I. Faruqui & L. Raschid-Sally). Pp. 41-58. Oxfordshire: CABI Publishing.

CDC (Centres for Disease Control). (2008). Shigellosis general information. [www document]. URL http://www.cdc.gov/. 4 December 2008.

Chambers, K. (2009). Zimbabwe's battle against cholera. *The Lancet*, **373**, 993–994.

Conter, M., Paludi, D., Zanardi, E., Ghidini, S., Vergara, A. & Ianieri, A. (2009). Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. *International Journal of Food Microbiology*, **128**, 497–500.

Cooke, F.J. & Shapiro, D.S. (2009). ProMED update: A review of emerging diseases reported on Pro-MED mail. Cholera in Zimbabwe. *International Journal of Infectious Diseases*, **13**, 119–120.

Cowling, R. (1991). Option for rural land use in Southern Africa: an ecological perspective. In: *A Harvest of Discontent: the Land Question in South Africa*. (Edited by M. de Klerk). Cape Town: Indara.

Croci, L., de Medici, D., Scalfaro, C., Fiore, A. & Toti, L. (2002). The survival of hepatitis A virus in fresh produce. *International Journal of Food Microbiology*, **73**, 29-34.

DEAT (Department of Environmental Affairs and Tourism) (2006). South Africa Environment Outlook. A report on the state of the environment. Department of Environmental Affairs and Tourism, Pretoria.

Dewedar, A. & Bahgat, M. (1995). Fate of Faecal Coliform bacteria in a wastewater retention reservoir containing *Lemna Gibba L*. *Water Research*, **29** (11), 2598-2600.

ECSCF (European Commission Scientific Committee on Food). (2002). Risk Profile on the Microbiological Contamination of Fruits and Vegetables Eaten Raw. European Commission Scientific Committee on Food. SCF/CS/FMH/SURF/Final. 29 April 2002.

Elizaquível, P. & Aznar, R. (2008). Comparison of four commercial DNA etraction kits for PCR detection of *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7, and *Staphylococcus aureus* in fresh, minimally processed vegetables. *Journal of Food Protection*, **71** (10), 2110-2114.

Elviss, N.C., Little, C.L., Hucklesby, L., Sagoo, S., Surman-Lee, S., de Pinna, E. & Threlfall, E.J. (2009). Microbiological study of fresh herbs from retail premises uncovers an international outbreak of salmonellosis. *International Journal of Food Microbiology*, 2009.

FAO (Food and Agricultural Organisation). (2005). AQUASTAT. Country profile South Africa. FAO, Rome. [WWW document]. URL: http://www.fao.org/ag/agl/aglw/aquastat/countries/index.stm. 3 October 2008.

Faruqui, N.I., Scott, C.A. & Raschid-Sally, L. (2004). Confronting the realities of wastewater use in irrigated agriculture: lessons learned and recomendations. In: *Wastewater Use in Irrigated Agriculture, Confronting the livelihood and environmental*

realities (edited by C.A. Scott, N.I. Faruqui & L. Raschid-Sally). Pp. 173-185. Oxfordshire: CABI Publishing.

Fayer, R., Gamble, H.R., Lichtenfels, J.R. & Bier, J.W. (1992). Waterborne and foodborne parasites. In: *Compendium of Methods for the Microbiological Examination of Foods* (Edited by Vanderzant, C. & Splittstoesser, D.F.). Pp. 789-809. Washington DC: American Public Health Association.

FDA (Food and Drug Administration) Center for Food Safety and Applied Nutrition. (1999). *Guidance for industry: Sampling and microbial testing of spent irrigation water during sprout production*. [WWW document]. URL http://www.cfsan.fda.gov/~dms/sprougd2.html. 20 March 2007.

Flowers, R.S., D'Aoust, J.Y., Andrews, W.H. & Bailey, J.S. (1992). *Salmonella*. In: *Compendium of Methods for the Microbiological Examination of Foods* (Edited by Vanderzant, C. & Splittstoesser, D.F.). Pp. 371-422. Washington DC: American Public Health Association.

Francis, G.A., Thomas, C. & O'Beirne, D. (1999). The microbiological safety of minimally processed vegetables. *International Journal of Food Science & Technology*, **34**, 1-22.

Gilboa, Y. & Friedler, E. (2008). UV disinfection of RBC-treated light greywater effluent: Kinetics, survival and regrowth of selected microorganisms. *Water Research*, **42**, 1043-1050.

Gill, M.I. & Selma, M.V. (2006). Overview of hazards in fresh-cut produce production: control and management of food safety hazards. In: *Microbial Hazard Identification in Fresh Fruits and Vegetables* (edited by J. James). Pp. 95-109. New Jersey: John Wiley & Sons.

Gleick, P.H. (2000). *The World's Water: The Biennial Report on Freshwater Resources*. Washington DC, Island Press.

Gómez, M., de la Rua, A., Garralón, G., Plaza, F., Hontoria, E. & Gómez, M.A. (2006). Urban wastewater disinfection by filtration technologies. *Desalination*, **190**, 16-28.

Green, K.Y., Chanock, R.M & Kapikian, A.Z. (2001). Human Caliciviruses. In: *Field Virology*, 4th ed. Pp. 841-873. Philadelphia: Lippincott, Williams & Wilkins.

Greig, J.D. & Ravel, A. (2009). Analysis of foodborne outbreak data reported internationally for source attribution. *International Journal of Food Microbiology*, **130**, 77 – 87.

Guévremont, E., Brassard, J., Houde, A., Simard, C. & Trottier, Y-L. (2006). Development of an extraction and concentration procedure and comparison of RT-PCR primer

systems for the detection of hepatitis A virus and norovirus GII in green onions. *Journal of Virological Methods*, **134**, 130–135.

Hamilton, A.J., Stagnitti, F.S., Premier, R. & Boland, A.M. (2006). Is the risk of illness through consuming vegetables irrigated with reclaimed wastewater different for different population groups? *Water Science & Technology*, **54** (11-12), 379-386.

Haramoto, E., Katayama, H., Oguma, K., Yamashita, H., Tajima, A., Nakajima, H. & Ohgaki, S. (2006). Seasonal profiles of human noroviruses and indicator bacteria in a wastewater treatment plant in Tokyo, Japan. *Water Science & Technology*, **54** (11), 301-308.

Harris, L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett., E.H. & Busta, F.F. (2003). Outbreaks associated with fresh produce: Incidence, growth and survival of pathogens in fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety*, **2**, 78-141.

Hurst, C.J., Crawford, R.L., Knudsen, G.R., McInerney, M.J. & Stetzenbach,L.D. (2002). Manual of Environmental Microbiology, 2nd ed. Washington, DC: ASM Press.

Hussain, I., Raschid, L., Hanjra, M., Marikar, F & Van der Hoek, W. (2001). *A Framework for Analysing Socio-economic, Health and Environmental Impacts of Wastewater Use in Agriculture in Developing Countries*, Working paper 26. International Water Management Institute (IWMI), Colombo, Sri Lanka, 31pp (As cited by Carr *et al.,* 2004).

ICD (International Classification of Diseases). (2000). Module 02, Lecture 01. Infectious foodborne pathogens. [WWW document]. URL http://www.icd-online.org/an/courspdf/WHO-ICD%20FSN%202000%20PDF/FS-0201.pdf. WHO, Geneva.

Jay, J.M. (1997). Do background microorganisms play a role in the safety of fresh foods? *Trends in Food Science & Technology*, **8**, 421-424.

Johnston, L.M. & Jaykus, L. (2004). Antimicrobial resistance of *Enterococcus* species isolated from produce. *Applied and Environmental Microbiology*, **70** (5), 3133–3137.

Kapp, C. (2009). South Africa heads into elections in a sorry state of health. *The Lancet*, **373**, 285–286.

Kautter, D.A., Solomon, H.M., Lake, D.E., Bernard, D.T. & Mills, D.C. (1992). *Clostridium botulinum* and its toxins. In: *Compendium of Methods for the Microbiological Examination of Foods* (Edited by Vanderzant, C. & Splittstoesser, D.F.). Pp. 605-621. Washington DC: American Public Health Association.

Kaysner, C.A., Tamplin, M.L. & Twedt, R.M. (1992). *Vibrio*. In: *Compendium of Methods for the Microbiological Examination of Foods* (Edited by Vanderzant, C. & Splittstoesser, D.F.). Pp. 451-473. Washington DC: American Public Health Association.

Koburger, J.A. & Wei, C. (1992). *Plesiomonas shigelloides*. In: *Compendium of Methods for the Microbiological Examination of Foods* (Edited by Vanderzant, C. & Splittstoesser, D.F.). Pp. 433-450. Washington DC: American Public Health Association.

Komnenic, V., Ahlers, R. & van der Zaag, P. (2009). Assessing the usefulness of the water poverty index by applying it to a special case: Can one be water poor with high levels of acess? *Physics and Chemistry of the Earth*, **34**, 219–224.

Koopmans, M. & Duizer, E. (2004). Foodborne viruses: an emerging problem. *International Journal of Food Microbiology*, **90** (1), 23–41.

Kothary, M.H. & Babu, U.S. (2001). Infective dose of foodborne pathogens in volunteers: a review. *Journal of Food Safety*, **21**, 49-73.

Madungwe, E. & Sakuringwa, S. (2007). Greywater reuse: A strategy for water demand management in Harare? *Physics and Chemistry of the Earth*, **32**, 1231-1236.

Malley, Z.J.U., Taeb, M., Matsumoto, T. & Takeya, H. (2009). Environmental sustainability and water availability: Analyses of the scarcity and improvement opportunities in the Usangu plain, Tanzania. *Physics and Chemistry of the Earth*, **34**, 3–13.

Mancini, G., Barone, C., Roccaro, P. & Vagliasindi, F.G.A. (2007). The beneficial effects of storage on the quality of wastewater for irrigation: a case study in Sicily. *Water Science & Technology*, **55** (1-2), 417-424.

Marcucci, M. & Tognotti, L. (2002). Reuse of wastewater for industrial needs: the Pontedera case. *Resources, Conservation and Recycling*, **34**, 249-259.

Matthews, K.R. (2006). Microorganisms associated with fruits and vegetables. In: *Microbiology of Fresh Produce* (edited by K.R. Matthews). Pp. 1-21. Washington DC: ASM Press.

McLain, J.E.T., Williams, C.F. (2008). Seasonal variation in accurate identification of *Escherichia coli* within a constructed wetland receiving tertiary-treated municipal effluent. *Water Research*, **42**, 4041-4048.

Midgley, G.F., Chapman, R.A., Hewitson, B. & Johnston, P. (2005). A status quo, vulnerability assessment of the physical and socio-economic effects of climate change

in the Western Cape. Report to the Western Cape Government, Cape Town, South Africa. CSIR Report No. ENV-S-C 2005-703.

NACMCF (National Advisory Committee of Microbiological Criteria for Foods) (1999). Microbiological safety evaluations and recommendations on sprouted seeds. *International Journal of Food Microbiology*, **52**, 123-153.

NDA (National Department of Agriculture). (2007). Agricultural Statistics. National Department of Agriculture, South Africa.

NFCS (National Food Consumption Survey. (2000). The National Food Consumptions Survey: Children aged 1-9 years, South Africa, 1999. Department of Health, South Africa.

Nieuwoudt, L. & Groenewald, J. (2003). *The Challenge of Change – Agriculture, Land and the South African Economy.* Pietermaritzburg, University of Natal Press.

NWRS (National Water Resource Strategy) (2004). First Edition, September 2004. Department of Water Affairs & Forestry, South Africa.

Okafu, C.N., Umoh,V.J. & Galadima, M. (2003). Occurrence of pathogens on vegetables harvested from soils irrigated with contaminated streams. *The Science of the Total Environment*, **311**, 49-56.

Oweis, T. & Hachum, A. (2009). Optimizing supplemental irrigation: Tradeoffs between profitability and sustainability. *Agricultural Water Management*, **96**, 511–516.

Parrot, L., Sotamenou, J., Kamgnia, B.D & Nantchouang, A. (2008). Determinants of domestic waste input in urban agriculture lowland systems in Africa: The case of Yaounde in Cameroon. *Habitat International*, doi:10.1016/j.habitatint.2008.08.002, Pp.1–8.

Parrot, L., Sotamenou, J. & Dia, B.D. (2009). Municipal solid waste management in Africa: Strategies and livelihoods in Yaounde, Cameroon. *Waste Management*, **29**, 986–995.

Qiao, G., Zhao, L. & Klein, K.K. (2009). Water user associations in Inner Mongolia: Factors that influence farmers to join. *Agricultural Water Management*, **96**, 822–830.

Raschid, L. (2004). IWMI assessment of wastewater irrigation practices in selected cities of less developed regions, Sri Lanka.

Rietveld, L.C., Haarhoff. J. & Jagals, P. (2009). A tool for technical assessment of rural water supply systems in South Africa. *Physics and Chemistry of the Earth*, **34**, 43–49.

Rose, J.B. (2007). Water reclamation, reuse and public health. *Water Science & Technology*, **55** (1-2), 275-282.

Savichtcheva, O. & Okabe, S. (2006). Alternative indicators of fecal pollution: Relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. *Water Research*, **40**, 2463-2476.

Schiemann, D.A. & Wauters, G. (1992). *Yersinia*. In: *Compendium of Methods for the Microbiological Examination of Foods* (Edited by Vanderzant, C. & Splittstoesser, D.F.). Pp. 433-450. Washington DC: American Public Health Association.

Schlech, W.F., Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haldane, W.V., Wort, A.J., Hightower, A.W., Johnson, S.E., King, S.H., Nichols, E.S. & Broome, C.V. (1983). Epidemic listeriosis – Evidence for transmission by food. *New English Journal of Medicine*, **308**, 203-206 (As cited by Beuchat, 1996b).

Schraft, H. & Watterworth, L.A. (2005). Enumeration of heterotrophs, faecal coliforms and *Escherichia coli* in water: comparison of 3M™ Petrifilm™ plates with standard plating procedures. *Journal of Microbiological Methods*, **60**, 335-342.

Scott, C.A., Faruqui, N.I. & Raschid-Sally, L. (2004). Wastewater use in irrigated agriculture: Management challenges in developing countries. In: *Wastewater Use in Irrigated Agriculture, Confronting the livelihood and environmental realities* (Edited by C.A. Scott, N.I. Faruqui & L. Raschid-Sally). Pp. 1-10. Oxfordshire: CABI Publishing.

Smith, J.L. & Buchanan, R.L. (1992). *Shigella*. In: *Compendium of Methods for the Microbiological Examination of Foods* (Edited by Vanderzant, C. & Splittstoesser, D.F.). Pp. 423-431. Washington DC: American Public Health Association.

Smith, M. & Shaw, R. (2007).Re-use of wastewater. Technical brief. WEDC Loughborough University, Leicestershire LE11 3TU UK. [WWW document]. URL http://info.lut.ac.uk/well/resources/technical-briefs/37-re-use-of-wastewater.pdf

Spinks, A.T., Dunstan, R.H., Harrison, T., Coombes, P. & Kuczera, G. (2006). Thermal inactivation of waterborne pathogenic and indicator bacteria at sub-boiling temperatures. *Water Research*, **40**, 1326-1332.

St. Louis, M.E. & Hess, J.J. (2008). Climate Change: Impacts on and implications for global health. *American Journal of Preventative Medicine*, **35** (5), 527–538.

Stats SA. (2005). General Household Survey – July 2005. [www document]. URL http://www.ukzn.ac.za/heard/resources/Stats/general%20household%20survey%20july%202005.pdf. 4 March 2009.

Stats SA. (2006). South African Statistics [www document] URL: http://www.ukzn.ac.za/heard/resources/Stats/SA%20Statistics%202006.pdf. 4 March 2009.

Stats SA. (2007). Labour Force Survey. [www document]. URL http://www.statssa.gov.za/publications/p0210/p0210March2007.pdf. 4 March 2009.

Steele, M. & Odumeru, J. (2004). Irrigation water as source of foodborne pathogens on fruit and vegetables. *Journal of Food Protection*, **67** (12), 2839 – 4901.

Stine, S.W.. (2005). Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce. *Journal of Food Protection*, **68** (5), 913–918.

Tempelhoff, J.W.N. (2009). Civil society and sanitation hydropolitics: A case study of South Africa's Vaal River Barrage. *Physics and Chemistry of the Earth*, **34**, 164–175.

Thornton, A. C., Jennings-Conklin, K. S., & McCormick, M. I. (2004). Noroviruses: agents in outbreaks of acute gastroenteritis. *Disaster Management and Response*, **2**, 4–9.

Toze, S. (2006). Reuse of effluent water – benefits and risks. *Agricultural Water Management*, **80**, 147-159.

UNAIDS (2004). 2004 Report on the Global AIDS epidemic. Joint United Nations Programme on HIV/AIDS. June 2004. [WWW document]. URL www.unaids.org/bangkok2004/GAR2004_html/GAR2004_33_en.htm. 10 October 2008.

USEPA (US Environmental Protection Agency). (1992). Guidelines for water reuse. EPA/625/R-04/108 (As cited by McLain & Williams, 2008).

Van der Hoek, W. (2004). A framework for a global assessment of the extent of wastewater irrigation: The need for a common wastewater typology. In: *Wastewater Use in Irrigated Agriculture, Confronting the Livelihood and Environmental Realities* (Edited by C.A. Scott, N.I. Faruqui & L. Raschid-Sally). Pp. 11-24. Oxfordshire: CABI Publishing.

Van Elfen, J. (2001). Cholera. In: *Dokter in die Huis* (Edited by Du Toit, D.). Pp. 108-109. Cape Town: Tafelberg Publishers.

Velazquez, L.C., Barbini, N.B., Escudero, M.E., Estrada, C.L. & de Guzman, A.M.S. (2009). Evaluation of chlorine, benzalkonium chloride and lactic acid as sanitizers for reducing *Escherichia coli* O157:H7 and *Yersinia enterocolitica* on fresh vegetables. *Food Control*, **20**, 262–268.

Venter, S.N., Steynberg, M.C., de Wet, C.M., Hohls, D., du Plessis, G. & Kfir, R. (1997). A situational analysis of the microbial water quality in a peri-urban catchment in South Africa. *Water Science & Technology*, **35** (11-12), 119-124.

Wadhwa, S.G., Khaled, G.H. & Edberg, S.C. (2002). Comparative Microbial Character of Consumed Food and Drinking Water. *Critical Reviews in Microbiology*, **28** (3), 249-279.

Welshimer, H.J. (1960). Survival of *Listeria monocytogenes* in soil. *Journal of Bacteriology*, **80**, 316-320 (As cited by Beuchat, 1996b).

WESGRO (2002). WESGRO factor sheet. [WWW document]. URL http://www.wesgro.org.za. 22 June 2007.

WESGRO (2003). Fruit sector fact sheet. Western Cape Trade and Investment Promotion Agency. Pp. 2-3.

WESGRO (2006). Fruit processing sector brief. Western Cape Trade and Investment Promotion Agency. Pp. 36-40.

WESGRO (2007). WESGRO homepage. [WWW document]. URL http://www.wesgro.org.za. 22 June 2007.

WESGRO (2008). About SA. [www document]. URL: http://www.wesgro.org.za/inde.php?view=article&catid=34%3Ainvest-in-the-wc. 3 December 2008.

WHO (World Health Organisation) (1975). Reuse of Effluent: Methods of Wastewater Treatment and Health Safeguards. *WHO Technical Report series No.517*, Geneva, Switzerland.

WHO (World Health Organisation) (1989). Health guidelines for the use of wastewater in agriculture and aquaculture. Geneva, Switzerland: World Health Organisation. Technical Report Series 778.

WHO (World Health Organisation) (1993). WHO Guidelines for Drinking Water Quality, 2nd ed. Recommendations, vol. 1. WHO, Geneva.

WHO (World Health Organisation) (2004). Water, Sanitation and Hygiene Links to Health – Facts and Figures updated November 2004. [WWW document]. URL http://www.who.int/water_sanitation_health/publications/facts2004/en/. 3 October 2008.

Yuk, H-Y., Yoo, M-Y., Yoon, J.W., Moon, K.D., Marshall, D.L. & Oh, D.H. (2006). Effect of combined ozone and organic acid treatment for control of Escherichia coli O157:H7 and Listeria monocytogenes on lettuce. *Journal of Food Science*, **71** (3), 83–87.

CHAPTER 3
AN EXPLORATORY STUDY OF THE QUALITY OF TWO WESTERN CAPE RIVERS AND THE INVESTIGATION OF THE MICROBIAL LEVELS OF IRRIGATION WATER AND IRRIGATED PRODUCE

SUMMARY

Two Western Cape rivers used for irrigation were chosen and evaluated at several sites over four months. The microbial community and chemical properties of the water was investigated and these findings were used to choose a site where the river water, irrigation water and irrigated produce could be examined. Studied organisms included amongst others *Salmonella*, *Listeria*, *E. coli* and intestinal *Enterococci*, while the chemical analysis consisted of pH, alkalinity, conductivity and chemical oxygen demand (COD). Faecal coliform counts as high as 460 000 organisms.100mL^{-1} and 1 600 000 organisms.100mL^{-1} was found in the Plankenburg and Mosselbank Rivers respectively, and although the irrigation water taken from the Mosselbank River seemed to be of a better microbial quality, counts as high as 1 600 000 organisms.100mL^{-1} was found on irrigated iceberg lettuce during the summer months. Potential human pathogens such as *E. aerogenes, E. cloacae*, and *E. coli* were frequently isolated from the water and produce. It was concluded that the river water was not suitable for use in irrigation practices as it regularly exceeded the limits for faecal coliforms as set out by the South African authorities (4 000 organisms.100mL^{-1}). Even though the irrigation water had a higher rate of compliance with the standards, potential human pathogens were occasionally isolated from these waters and in many cases the same organisms were isolated from the irrigated produce, showing that there is a definite carry-over of pathogens from irrigation water to irrigated produce.

INTRODUCTION

A sharp increase in the number of foodborne outbreaks have been witnessed globally over the last decade (Yuk *et al*., 2006; Allende *et al*., 2009, Greig & Ravel, 2009; Velazquez *et al*., 2009). Possible human pathogens such as *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, *Clostridium botulinum* and *Staphylococcus aureus* have been linked to foodborne disease outbreaks in the USA between 1993 and 1997 (Wadhwa *et al*., 2002) and all of these have in the past been isolated from various types of fresh produce (Beuchat, 1996). One of the factors thought to contribute to this phenomenon is

the use of contaminated water for the irrigation of minimally processed foods. In South Africa unserviced informal settlements on the banks of rivers and the failing sewage systems and water treatment works are given as the main routes of contamination of these waters.

During 2004, faecal coliform counts as high as 17 420 000 organisms.100 mL^{-1} were recorded from the Plankenburg River just after its confluence with the Eerste River (Dr. Jo Barnes, 2008, University of Stellenbosch, unpublished data). This water is used for the irrigation of fruits and vegetables by farmers downstream of this point, and especially during the dry summer months when irrigation is crucial, farmers have no choice but to use this contaminated water if they want to be sure of a good harvest.

Guidelines for the use of contaminated water for irrigation purposes are available and these are influenced by various factors such as the specific products to be irrigated. The South African Department of Water Affairs and Forestry states that, for the irrigation of crops eaten raw or minimally processed, faecal coliform counts exceeding 1 000 organisms.100mL^{-1} places farm workers, food handlers and consumers at risk of contracting a foodborne illness (DWAF, 1996a). The Department of Health sets the microbial guidelines for fruit and vegetables eaten raw at less than 200 coliforms per gram and zero *E. coli* per gram of product, while *Salmonella* species should also be absent in 25 g of the sample tested (DoH, 2006). The question arises that, if river water with levels of contamination exceeding those of the guidelines is used for the irrigation of produce intended to be eaten raw, what effect will this have on the microbial quality of the product and on the health of consumers eating these products? In short: Is there a carry-over of pathogens from the irrigation water onto the product?

To find a possible answer to this question an exploratory study of the types and quantities of the microbial contaminants present in a representative sample of river water will be done. These organisms include coliforms, faecal coliforms, *E. coli*, *Salmonella*, *Listeria*, *Staphylococcus*, intestinal *Enterococci*, aerobic and anaerobic spore formers and the heterotrophic count. Produce sites will be chosen and a study of the types and quantities of the abovementioned microbes present on produce irrigated with contaminated river water will follow. The data collected from the river water, irrigation water and the irrigated produce will then be compared to determine whether a carry-over of organisms does take place.

MATERIALS AND METHODS

Site description, selection criteria and maps

In order to find suitable sites where the project could be executed, certain factors were taken into consideration when potential sites were investigated. The criteria included: the types and pathways of contamination, the type of farming and the crops produced, the sources of irrigation and the irrigation technologies employed, and the availability of the site to be monitored over an extended period. After all the potential sites were visited, the four most suitable ones were chosen and sampled approximately every three weeks for the duration of the baseline river study.

Plankenburg site 1 – This site is situated just downstream of the Plankenbrug industrial area and the Kayamandi informal settlement (Fig. 1). Here, faecal contamination enters the river because of the lack of sanitary services in the informal settlement. Where sanitation is present, it is often not in a proper working condition and individuals have no other choice as to use the river for the disposal of human waste (J.M. Barnes, 2007, personal communication; Britz *et al*., 2007). Additionaly the Krom River runs through Stellenbosch and brings stormwater and run-off to the Plankenburg at their confluence just up-stream of this sampling point. Especially in the dry summer months, this river is used by farmers downstream to irrigate an array of produce including grapes, citrus fruit, strawberries, pears and green beans.

Plankenburg site 2 – At the confluence of the Plankenburg- and Eerste Rivers (Fig. 1), samples were taken on the bank of the Plankenburg side of the confluence. This site is downstream from Plankenburg site 1, and thus the sources of contamination would be more or less the same. As mixing of the two rivers does occur, the quality of the water from the Eerste River may have an impact on the levels of contamination found at this point. The water from this site is used for irrigation purposes downstream and most of the irrigated produce, including grapes, citrus fruit and pears, are destined for the export market (J. M. Barnes, 2007, personal communication).

Plankenburg site 3 – After their confluence, water from the Plankenburg and Eerste Rivers are fed into a cement canal from which downstream farmers pump water into their irrigation dams. From these dams the water is then used for irrigation as required during the dry summer months. At this site (Fig. 1) the samples were taken from the canal as it

enters an irrigation dam. On this specific farm the water is used for the irrigation of grapes, pears and vegetables such as green beans.

Mosselbank site 4 – This site is situated North West of the Kraaifontein sewage works, approximately one kilometre downstream of the discharge point of the treatment works. The discharge, together with storm water run-off from the nearby residential area, flows into a cement furrow which feeds into a branch of the Mosselbank River further downstream. Samples were taken from a sandy patch in this branch before its confluence with the Mosselbank River – the exact location from where a large-scale commercial farmer pumps water for the overhead irrigation of various vegetables during periods of water scarcity. During the dry summer months the produce is irrigated for an hour every afternoon. Apart from the samples taken from the furrow, water from the irrigation dam and samples of the irrigated produce were evaluated.

Sampling methods

River and irrigation water – Guidance on sampling of rivers and streams as given by SANS 5667-6 (2006) was used as guideline for the sampling of river and irrigation water. Safety measures, such as wearing surgical gloves and gloves which extend above the elbows, and waders or gumboots, were always taken. Care was taken not to disturb the sediment, and a sample was taken as near to the middle of the river as possible. A sterile, one litre Schott bottle was submerged to a depth of 30 cm under the surface pointing toward the direction of flow, before the cap was removed and the bottle filled. The bottle was completely filled and the cap replaced before it was taken out of the water. The bottles were put on ice in a cooler bag and analysed within four to six hours after sampling. Care was taken not to contaminate any surrounding surfaces, and all sampling equipment and protective clothing were disinfected before return to the laboratory.

Vegetables – The chosen irrigated produce consisted of cabbage heads (*Brassica oleracea*) and iceberg lettuce (*Lactuca sativa*). Always wearing gloves and gumboots, vegetables were picked five rows into the field to ensure that factors such as water splashing onto the produce from the wheels of tractors were eliminated. A sprinkler was chosen and two samples were taken – one metre to each side of the sprinkler (at 180° of each other). The samples were placed into sterile polypropylene bags, placed in a cooler bag and analysed within four to six hours. Upon analysis, three layers of leaves were

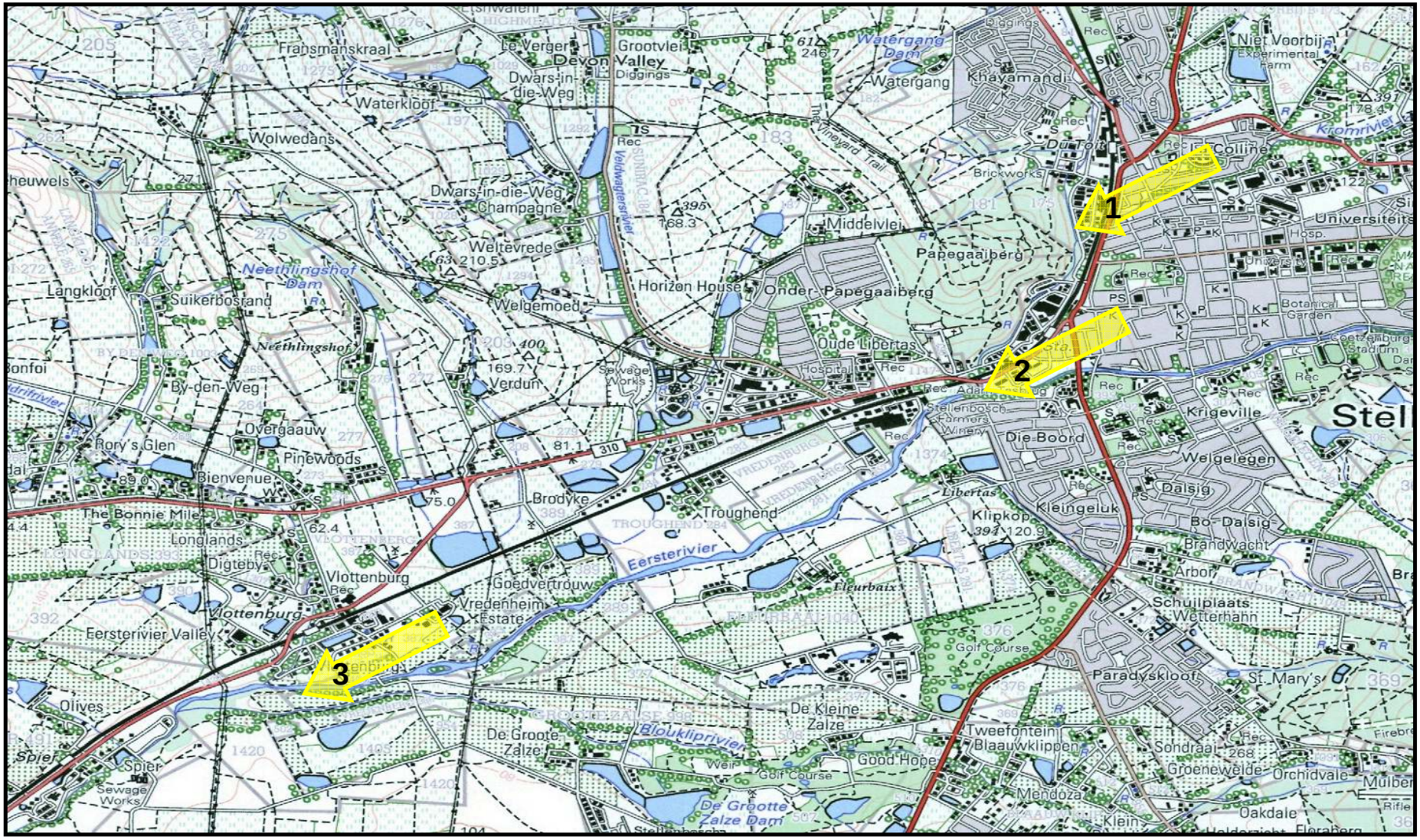

Figure 1 Location of sampling sites in the Plankenburg River, Stellenbosch (Map, 2003).

aseptically removed and discarded before the forth layer of leaves was removed and placed in a sterile stomacher bag. After 300 mL of sterile physiological saline solution (PSS: 0.85% NaCl) had been added, the bag was sealed and shaken carefully for two min to wash the leaves. The "wash-water" was then placed in a sterile Schott bottle and analysed in the same manner as the river and irrigation water. Vegetables were picked in accordance with the harvesting plan of the farmer to ensure that the produce was representative of produce to be delivered to the fresh produce market.

Microbial enumeration

Aerobic Colony Count – The water or "wash-water" sample was shaken vigorously before 1 mL was used to prepare a dilution series (10^{-1} to 10^{-8}). This was done using eight McCartney bottles each containing 9 mL of sterile PSS. One millilitre of each dilution was carried over to each of two petri dishes (90 mm diameter) to facilitate duplicate analyses. Pour plates were prepared using 25 mL of Plate Count Agar (Merck), and the petri dishes were rotated in a figure of eight to homogenise the mixture and then left to set. Once the PCA had set, the plates were covered with a layer of Bacteriological Agar (Merck) and were once again allowed time to set. The inverted plates were incubated at 30ºC for 72 h before enumeration. Only plates with between 25 and 250 colonies were counted, and the number of organisms per millilitre calculated using the correct dilution factor from selected pour plates (ISO, 1991).

Aerobic and Anaerobic Spore-formers – A test tube containing 18 mL of PSS was inoculated with 2 mL of undiluted sample and closed. A thermometer was placed in a second tube containing 20 mL of PSS, and both the tubes were placed in a waterbath at 75ºC (MFLP 44, 1998). Once the thermometer indicated that the mixture within the tubes was at 75ºC, it was left for 20 min before the tubes were taken out and allowed to cool down. The tube containing the sample was then used to prepare a dilution series (10^{-1} to 10^{-8}) as described previously. One millilitre of each dilution was carried over to each of two petri dishes, one for aerobic and one for anaerobic incubation, respectively, and pour plates were prepared using 25 mL Trypticase Soy Agar (TSA) (Merck). The plates were allowed to set before the aerobic plates were inverted and incubated at 35ºC for 48 h. The anaerobic plates were inverted, placed in an AnaeroJar (Oxoid) along with a strip of AnaeroCult A (Merck) and incubated in the same way. Only plates with between 25 and 250 colonies were taken into consideration for enumeration, and the number of aerobic and anaerobic organisms was calculated using the correct dilution factor.

Coliforms, Faecal coliforms and E. coli – These organisms were cultivated using the multiple tube fermentation (MTF) method, and enumerated by means of the appropriate MPN tables. Ten millilitres of double strength Lauryl Sulfate Tryptose (LST) broth (Merck) was transferred into each of five tubes containing a Durham tube, while 10 mL of single strength LST was transferred to each of 45 test tubes, also containing a Durham tube. The media was sterilised as stipulated by the manufacturer and allowed to cool. After sterilisation the tubes were examined to ensure no gas bubbles were trapped in the Durham tubes (MFHPB 19, 2002).

A one millilitre aliquot of the undiluted sample was used to prepare a dilution series (10^{-1} to 10^{-8}) as described previously. Each of the five tubes containing the double strength medium and each of five tubes containing the single strength medium were inoculated with 10 mL and 1 mL of the undiluted sample, respectively. Of the remaining single strength LST tubes, five tubes were inoculated with one millilitre of each of the dilutions in the series. The tubes were incubated at 35ºC for 24 h before examination. If no gas was present in the Durham tubes, the tubes were incubated for a further 24 hours. Each of the gas-positive tubes was documented before a loop-full of this media was transferred to a tube containing 10 mL of sterile Brilliant Green Lactose Bile (BGLB) broth (Merck) and a Durham tube.

The inoculated tubes were incubated at 35ºC for 24 h before examination for gas production. The gas-negative tubes were incubated for a further 24 hours, while the gas positive tubes were documented and loop-fulls were transferred to tubes containing 10 mL of sterile *Escherichia coli* (EC) broth (Merck) and a Durham tube.

After incubation in a waterbath at 44.5ºC, the tubes were once again examined for gas production. The gas-positive tubes were noted and streaked out on pre-dried L-EMB (Levine Eosin Methylene Blue) plates (Merck) and incubated at 35ºC while the gas-negative tubes were incubated for a further 24 h and again examined for gas production. After 24 h, the LEMB-plates were inspected for growth.

Typical *E. coli* colonies were described as having a metallic green sheen (Merck, 2005). Presumptive positive colonies were subjected to further tests and confirmed to be *E. coli* if they were Gram-negative, non-sporing, oxidase-negative and catalase-positive rods (MFHPB 19, 2002).

Staphylococci – The surface of a solid selective plate was inoculated with the water or "wash-water" to enumerate and identify different species of coagulase-positive Staphylococci (SABS 6888-1, 1999) present in the sample.

Baird-Parker medium (BP) was prepared by sterilising 950 mL of Baird-Parker Agar (Merck) and allowing it to cool down to 50ºC before adding 50 mL of Egg-Yolk Tellurite Emulsion (Oxoid). The plates were poured, allowed to set, and kept in the refrigerator until needed. McCartney bottles each containing 9 mL of PSS were used to prepare a dilution series as described previously. A sterile pipette was used to transfer 0.1 mL aliquots of each of the dilutions to a pre-dried BP plate. The inoculum was spread over the surface of the plate using a flamed glass hockey stick. The plates were set aside to dry for 15 min before they were inverted and incubated at 35ºC. After 24 h the plates were examined for growth and the colonies marked before returning them to the incubator. After another 24 h the plates were again examined and the morphology and the number of colonies present were documented. Only plates with between 25 and 250 colonies were taken into account for enumeration. Typical colonies were black and differ in size, with *S. aureus* forming a clear halo in the media surrounding the colony (Merck, 2005). Presumptive positive colonies were subjected to further tests and confirmed to be Staphylococci if they were Gram-positive, catalase-positive and oxidase-negative cocci (SABS 6888-1, 1999).

Salmonella – The identification of *Salmonella* species requires the pre-enrichment and enrichment of the inoculum before it can be plated out for identification (SABS 6579, 2004).

Buffered Peptone Water (225 mL) (Merck) was inoculated with 25 mL of undiluted test sample and incubated at 35ºC. After 16 to 20 h volumes of 0.1 mL and 10 mL, respectively, of the pre-enriched sample was used to inoculate 10 mL of Rappaport Vasiliadis (RV) broth (Merck) and 100 mL of Selenite Cystine (SC) broth (Merck). The RV broth was incubated at 42ºC for 24 h, while the SC broth was incubated at 35ºC for the same period of time. Each of these cultures was subsequently streaked out on pre-dried Xylose Lysine Deoxycholate (XLD) plates (Merck) before incubation for a further 24 h. The plates were inverted and incubated at 35ºC for 24 h. After the second 24 h incubation period, the cultures were again streaked out on pre-dried XLD plates. These plates were inverted and incubated as described above. After incubation all the plates were examined for growth and colony morphology and any changes in the colour of the media were documented. Typical *Salmonella* colonies are colourless with a black centre and changes in the colour of the media may occur (Merck, 2005). Positive organisms were taken as

those which were Gram-negative bacilli, catalase-positive, oxidase-negative non-lactose fermenters (SABS 6579, 2004).

Listeria – The detection of *Listeria spp*. necessitates primary and secondary enrichment of the test sample before it can be plated out and colonies can be identified (SABS 11290-1).

Half strength Frazer broth (9 mL) (Merck) was inoculated with 1 mL of the undiluted water sample before incubation at 30ºC. After 24 h, 0.1 mL of this suspension was used to inoculate 10 mL of full strength Frazer broth (Merck). A loop full of the suspension was streaked out on a pre-dried Oxford (Merck) and PALCAM (Merck) plate, respectively. The plates were inverted and incubated micro-aerobically at 35ºC. The full strength Frazer suspension was incubated at 35ºC for 48 h before it was also streaked out on Oxford and PALCAM media and incubated. All the plates were examined for growth after 24 h. If the growth was slight, the plates were incubated for a further 24 h. The morphology and changes in the colour of the media were noted, and colonies were subjected to further biochemical tests for identification. Typical colonies on Oxford agar are described as greyish colonies, about 1 mm big, surrounded by a black halo in the media after 24 h of growth. After 48 h, the colonies become darker with a possible greenish sheen, they have a sunken centre and a black halo in the surrounding media (Merck, 2005). After 24 h of growth on PALCAM agar, colonies were about 1.5 – 2 mm in diameter with a greyish green to olive green colour, sometimes with black centres, but always with black halo's. After 48 h, the colonies remain more or less the same size, but turn completely green with sunken centres and surrounded by a black halo (Merck, 2005). *Listeria spp*. are Gram-positive rods, but can also be in cocci form. These organisms are catalase-positive and oxidase-negative and exhibit a tumbling motility at 25ºC (Merck, 2005).

Intestinal Enterococci – Enumeration is done by means of filtration before the filter is placed on a selective medium containing 2,3,5-triphenyltetrazolium chloride (SANS 7899-2, 2004).

The selective medium was made up by preparing and sterilising 1 L of Slanetz and Bartley Agar (Merck) before leaving it to cool down to 60ºC. Meanwhile, 1 g of 2,3,5-triphenyltetrazolium chloride was dissolved in 100 mL distilled water. The solution was sterilised by filtration through a 0.2 µm filter before 10 mL was added to the litre of prepared media. The plates were poured, left to set and were kept at 4℃ until used. The undiluted sample (100 mL) was filtered aseptically through a 0.45 µm membrane filter. The filter was transferred to the surface of a pre-dried Slanetz and Bartley plate ensuring

that the whole filter made contact with the surface of the plate which was subsequently incubated at 35ºC for 44 h. After incubation, the filter was examined for growth and aseptically transferred to the surface of a Bile Esculin Azide plate (Merck) which had been preheated to 44ºC. After a two hour incubation period the plate was examined for growth, and colonies showing a tan to black colour in the surrounding medium were counted as intestinal enterococci. These colonies were confirmed positive if they were Gram-positive cocci (Merck, 2005).

Chemical analysis

The following characteristics of the river and irrigation water were monitored according to Standard Methods (APHA, 1998): pH, alkalinity and chemical oxygen demand (COD). Conductivity was determined using a Hanna Instruments (HI8733) conductivity meter. The COD was determined colorimetrically using a DR2000 spectrophotometer (Hach Co. Loveland, CO) and standardised procedures (APHA, 1998).

RESULTS AND DISCUSSION

Baseline river data

For the baseline study of the Plankenburg and Mosselbank Rivers sites best suited to sample the river water, irrigation water and irrigated produce were chosen. Four sites (three in the Plankenburg and one in the Mosselbank River) were chosen as described earlier. Sampling took place over a period of four months to gather data for the baseline study.

Chemical parameters – The chemical analyses of the river water samples are summarised in Table 1. As was expected, the temperature of the water increased during the months (December and February) in which the ambient temperatures were also higher. The water temperature at site 1 ranged between 12.5℃ in September and 20.8℃ in February, while the pH increased from 5.97 to 6.80 for the same period. According to DWAF (1996a), an increase in water temperature shows a relationship with an increase in pH and therefore this may offer a possible explanation for the slightly higher pH during the warmer months. Although the pH of the samples taken from Site 1 only fell within with the Target Water Quality Range (TWQR) for irrigation and recreational waters once, the water is still grouped as “generally safe to use for irrigation and recreational purposes where chemical parameters are concerned” by the South African Water Quality Guidelines (DWAF, 1996a; DWAF, 1996b). According to these guidelines, water of an acceptable quality will have a

pH ranging between 6.5 and 8.5. No relationship between either of these parameters and the COD, alkalinity or conductivity of the samples could be seen. The alkalinity ranged between 50 and 125 mg $CaCO_3.L^{-1}$, while the conductivity of the samples ranged between 20 and 55 $mS.m^{-1}$. COD values of between 40 and 66 $mg.L^{-1}$ were noted during the sampling period.

As with Site 1, a relationship between the water temperature and the pH values could be seen for Site 2 (Plank 2). As the temperature increased from 12.5℃ in September to 21.6℃ in February, the pH increased from 6.38 to 6.85. Again, the pH did not always fall within the TWQR for irrigation or recreational waters, but it was still acceptable to use for recreational purposes and in agricultural application (DWAF, 1996a; DWAF, 1996b). No specific relationship could be seen between the alkalinity, conductivity and COD values. While the alkalinity ranged between 37 and 350 mg $CaCO_3.L^{-1}$, the conductivity varied between 22 and 75 $mS.m^{-1}$. The COD values ranged from 7 to 73 $mg.L^{-1}$

The relationship between pH and temperature was seen for a third time at Plank 3 when the pH increased from 6.24 to 7.33. Over the same period the temperature increased from 15.7℃ to 26.4℃. The water was still considered suitable for recreational and irrigation purposes although it only complied with the TWQR once during the sampling period. While the alkalinity ranged between 25 and 100 mg $CaCO_3.L^{-1}$, conductivity values of between 49 and 72 $mS.m^{-1}$ were noted. COD values varied between 53 and 193 $mg.L^{-1}$.

At Site 4, the site in the Mosselbank River, the relationship between temperature and pH could again be seen as the pH increased from 6.56 to 6.81 as the temperature increased from 13.6℃ to 22.8℃. The pH value of this water fell within the TWQR limits throughout the sampling period and could thus be taken as suitable for use in agricultural and recreational applications. The alkalinity and conductivity values for this site were higher than found for the Plankenburg River, ranging between 150 and 1500 mg $CaCO_3.L^{-1}$ and 77 and 91 $mS.m^{-1}$, respectively. The COD values were noted to be more or less the same as those found in the Plankenburg River, ranging between 25 and 45 $mg.L^{-1}$.

Microbial enumeration – The water samples were subjected to microbiological tests and the following microbial data (Table 2) was recorded. The Aerobic Colony Count (ACC) found at Plank 1 ranged between 8 800 $cfu.mL^{-1}$ in October 2007 and 150 000 $cfu.mL^{-1}$ in December 2007. Although the main focus of this project was to determine if a link

between faecally polluted irrigation water and contaminated crops exists, it is still relevant to mention that the water of the Plankenburg River is used for domestic purposes by communities in informal settlements along the riverbanks. DWAF sets the TWQR for heterotrophic bacteria in water for domestic use between 0 and 100 cfu.mL^{-1}, while counts of more than 1 000 cfu.mL^{-1} is seen to have an increased risk for the transmission of disease (DWAF, 1996c). Apart from this, a heterotrophic bacterial count exceeding 50 000 cfu.mL^{-1} is one of the main causes of clogging of drip irrigation systems (DWAF, 1996a).

For this site, counts of aerobic and anaerobic sporeformers peaked at 40 and 70 cfu.mL^{-1}, respectively, while instances where zero sporeforming organisms were detected occurred. It should be noted that if an organism is not detected, it is not to say that it is not present! It is possible that the organism could be in a non-culturable state or the levels were to low to determine.

The coliform counts for Plank 1 ranged between 17 000 and 460 000 org.100 mL^{-1} during the sampling period, while faecal coliform counts of between 14 000 and 160 000 cfu.100 mL^{-1} were noted. According to the guidelines for irrigation and recreational waters (DWAF, 1996a; DWAF, 1996b), more than 2 000 faecal coliforms per 100 mL water can be seen as having an increased risk for the transmission of disease. Typical *E. coli* colonies were isolated from all samples taken from this site, and if it is assumed that all of the faecal coliforms present in the samples were *E. coli*, the guidelines advise that humans must not come into contact with this water and that it is definitely not suitable for the irrigation of foodstuffs (DWAF, 1996a)

Table 1 Chemical analysis of water taken from the Plankenburg and Mosselbank Rivers.

Sampling date	**Temperature** (℃)	**pH**	**Alkalinity** (mg $CaCO_3.L^{-1}$)	**Conductivity** ($mS.m^{-1}$)	**COD** ($mg.L^{-1}$)
Plank 1					
Sept 2007	12.5	5.97	100	47	40
Oct 2007	17.9	6.41	125	55	66
Dec 2007	19.6	6.47	50	39	54
Feb 2008	20.8	6.80	65	20	40
Plank 2					
Sept 2007	12.5	6.38	350	45	51
Oct 2007	18.2	6.20	125	75	7
Dec 2007	19.9	6.78	37	22	73
Feb 2008	21.6	6.85	122	36	41
Plank 3					
Sept 2007	15.7	6.24	50	72	193
Oct 2007	17.9	6.03	75	49	81
Dec 2007	18.7	6.33	100	49	101
Feb 2008	26.4	7.33	25	72	53
Mossel 4					
Sept 2007	13.6	6.56	150	91	45
Oct 2007	20.1	6.63	1500	83	37
Dec 2007	22.8	6.81	175	77	25

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Table 2 Microbial loads of water from the Plankenburg and Mosselbank Rivers sampled between September 2007 and February 2008.

Sampling Date	ACC (cfu.mL^{-1})	A^{+}spores (cfu.mL^{-1})	A^{-}spores (cfu.mL^{-1})	Coliforms (org.100mL^{-1})	Faecal coli (org.100mL^{-1})	*E. coli* (cfu.100mL^{-1})	*Salmonella*	*Listeria*	*Staph* (cfu.mL^{-1})	*Enterococci* (cfu.100mL^{-1})
Plank 1										
Sept 2007	53000	40	10	70000	14000	TG	TG	-	80	20
Oct 2007	8800	10	30	17000	17000	TG	TG	TG	380	5
Dec 2007	150000	ND	ND	460000	160000	TG	TG	TG	ND	TNTC
Feb 2008	25900	ND	70	350000	28000	TG	ND	TG	390	65
Plank 2										
Sept 2007	41000	20	10	54000	9500	TG	TG	-	40	254
Oct 2007	12000	ND	10	54000	54000	TG	ND	TG	TNTC	TNTC
Dec 2007	22800	ND	ND	540000	8100	TG	TG	TG	ND	67
Feb 2008	9400	ND	10	4900	22000	TG	TG	TG	3100	7
Plank 3										
Sept 2007	7000	10	ND	610	23	TG	TG	-	ND	19
Oct 2007	61000	30	ND	3500	2100	TG	TG	ND	3	15
Dec 2007	11000	ND	ND	9200	ND	TG	TG	TG	1	ND
Feb 2008	9200	ND	ND	1400	7000	ND	TG	TG	4500	16
Mossel 4										
Sept 2007	21000	ND	ND	46000	13000	TG	ND	TG	10	24
Oct 2007	66000	ND	ND	460000	460000	TG	TG	TG	296	ND
Dec/2007	11500	ND	ND	92000	4000	TG	TG	TG	ND	267

ND = None detected; TG = Typical growth; ACC = Aerobic Colony Count, A^{+} = Aerobic Sporeformers, A^{-} = Anaerobic Sporeformers.

Of the samples taken from the Plank 1 site, both typical colonies of *Salmonella spp.* and *Listeria spp*. were noted. Staphylococci counts ranged from none detected to 390 cfu.mL^{-1} but no guideline for the risk of transmission of these organisms could be found. As this river is sometimes used for recreational purposes, it is important to note that the samples taken during December 2007 and February 2008 could be considered as a slight risk of transmission of infection for the consumer as the number of faecal enterococci exceeded the TWQR of between zero and 30 cfu.mL^{-1} (DWAF, 1996b).

The water samples taken at Plank 2 can be considered as unfit for domestic use as the ACC counts ranged between 9 400 and 41 000 cfu.mL^{-1} (Table 2). This water can, however, be used for drip irrigation purposes as clogging of irrigation systems at these levels of contamination are rare (DWAF, 1996a). The levels of aerobic and anaerobic sporeformers were found to range between zero and 20 and 10 cfu.mL^{-1}, respectively. The amount of coliform bacteria present in these samples varied between 4 900 and 540 000 cfu.100 mL^{-1}, while faecal coliform counts of between 8 100 and 54 000 org.100 mL^{-1} were noted. Once again, this water is only suitable for the irrigation of fodder, trees and parks as levels exceeding 1000 faecal coliforms per 100 mL of water were found (DWAF, 1996a). Typical *E. coli* colonies were isolated on all four occasions indicating a high likelihood of infection if this water was used for the irrigation of minimally processed foods. On three of the four sampling occasions typical colonies of *Salmonella spp*. and *Listeria spp*. were found, highlighting the possibility of infection if this water was to be used for the irrigation of foods to be eaten raw. Staphylococci counts peaked at 3 100 cfu.mL^{-1}, while faecal enterococci counts varied between 7 and 254 cfu.mL^{-1}.

At Plank 3, as expected after the confluence of the polluted Plankenburg River with the cleaner Eerste River, lower numbers of most of the microbes were noted. ACC counts varied between 7 000 and 61 000 cfu.mL^{-1}, which was still enough to cause possible problems if drip irrigation systems were used. Aerobic sporeformers peaked at 30 cfu.mL^{-1}, while no anaerobic sporeformers were detected. In comparison with the other two sites in the same river, considerably lower counts of coliforms and faecal coliforms were noted. These peaked at 9 200 and 7 000 org.100 mL^{-1}, respectively. Although there was a decrease in the level of contamination, these levels still exceed the TWQR for faecal coliforms in irrigation water (DWAF, 1996a) and therefore it is best not used for the irrigation of foodstuffs. Nonetheless, typical colonies of potential human pathogens *Listeria, E. coli* and *Salmonella* were isolated from this site showing that this water can still be a route of possible transmission of infectious diseases. Staphylococci counts peaked at 4 500 cfu.mL^{-1} – higher than the levels found at the other sites. A possible explanation

could be that these organisms thrive in habitats where lower levels of *E. coli* are present (J.M. Barnes, 2008, personal communication).

At site 4 in the Mosselbank River, high levels of microbial contamination – especially faecal coliforms - were found. Although the ACC counts ranged between 11 500 and 66 000 cfu.mL^{-1}, this is not too relevant as this water is not used for domestic purposes like the water from the Plankenburg River was. These levels of contamination could, however, cause problems if the farmer makes use of drip irrigation. Aerobic and anaerobic sporeformers were not detected in these waters. Levels of coliform bacteria ranged between 46 000 and 460 000 org.100 mL^{-1}, while faecal coliform levels of between 4 000 and 460 000 org.100 mL^{-1} were noted. According to the guidelines for irrigation water (DWAF, 1996a) these levels of faecal coliforms are as much as 460 times higher than the levels already described to be unfit for the irrigation of foodstuffs. Furthermore, typical *Salmonella* colonies were isolated from two of the three water samples, while typical colonies of *E. coli* and *Listeria* were isolated from all of the samples. Staphylococci counts of as high as 296 cfu.mL^{-1} were found, although these organisms were sometimes not detected. Faecal enterococci counts varied between none detected to 267 cfu.mL^{-1} over the sampling period.

To illustrate how the water from these rivers complied with the guidelines for irrigation water as stipulated by DWAF (1996a), and the guidelines for recreational waters (DWAF, 1996b) the total coliform and faecal coliform data is represented in Figs. 2 - 5.

Of the samples taken from the river at Plank 1, the faecal coliform counts did not once comply (Fig. 2) to the South African guidelines for irrigation water. During the warmer months (December and February) the faecal coliform counts reached levels as high as 160 000 and 28 000 org.100 mL^{-1}, respectively indicating that warmer water temperatures could favour the growth of these organisms. As these organisms are inhabitants of the gastrointestinal tract of mammals and generally proliferate at 37℃, this could possibly offer an explanation for this phenomenon. Situated closest to the possible sources of contamination, namely the Kayamandi Informal Settlement and the Plankenbrug Industrial area, it was expected that the higher faecal coliform counts (160 000 org.100 mL^{-1}) would be found here.

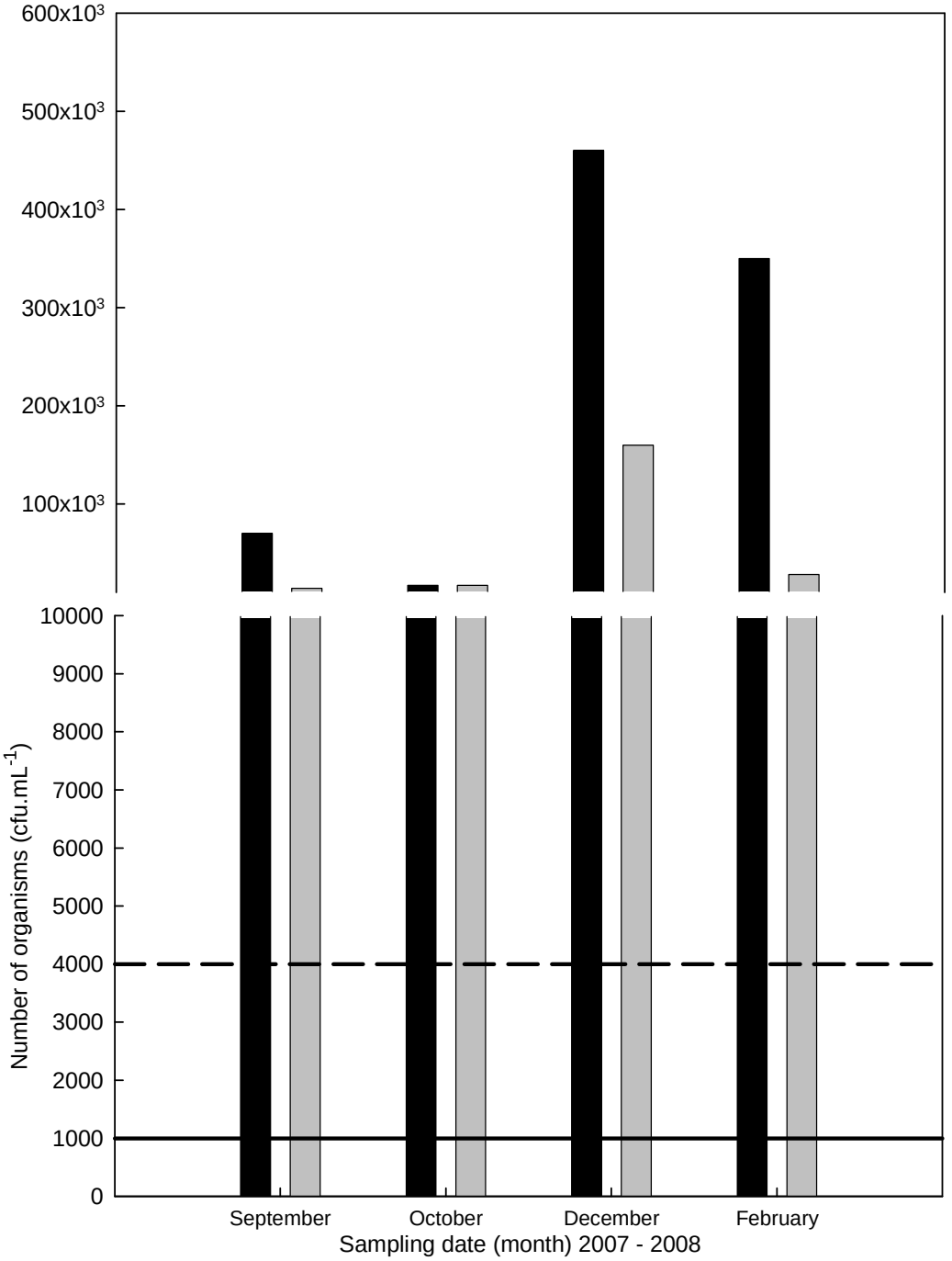

Figure 2 Compliance of river water from Plank 1 site with the South African guidelines (DWAF, 1996b; DWAF, 2008), in terms of coliform (black) and faecal coliform (grey) counts in water used for recreational purposes or the irrigation of foods eaten raw or minimally processed (Dotted line = DWAF guideline; solid line = WHO guideline (WHO, 1989)).

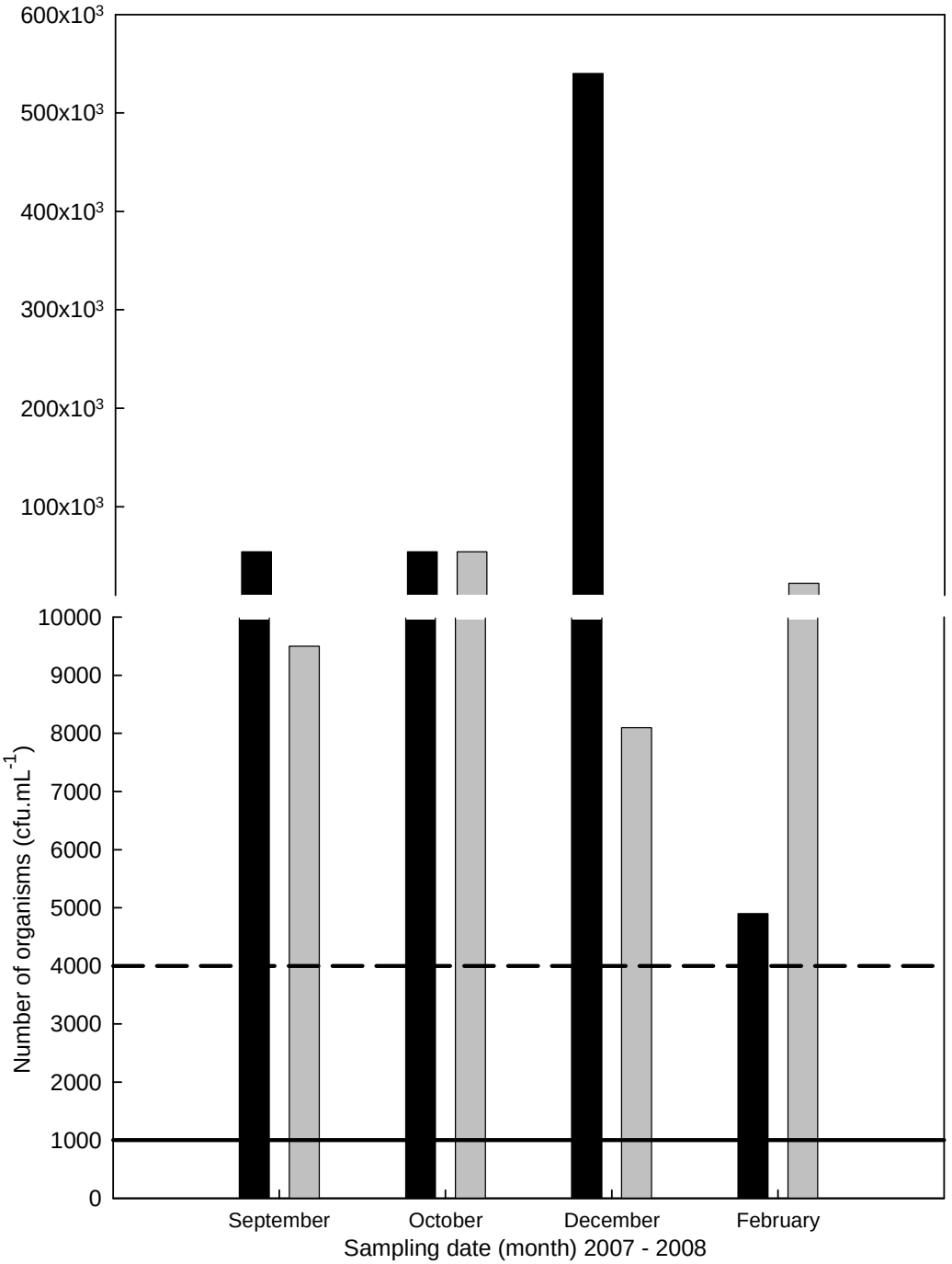

Figure 3 Compliance of river water from Plank 2 site with the South African guidelines (DWAF, 1996b; DWAF, 2008), in terms of coliform (black) and faecal coliform (grey) counts in water used for recreational purposes or the irrigation of foods eaten raw or minimally processed (Dotted line = DWAF guideline; solid line = WHO guideline (WHO, 1989)).

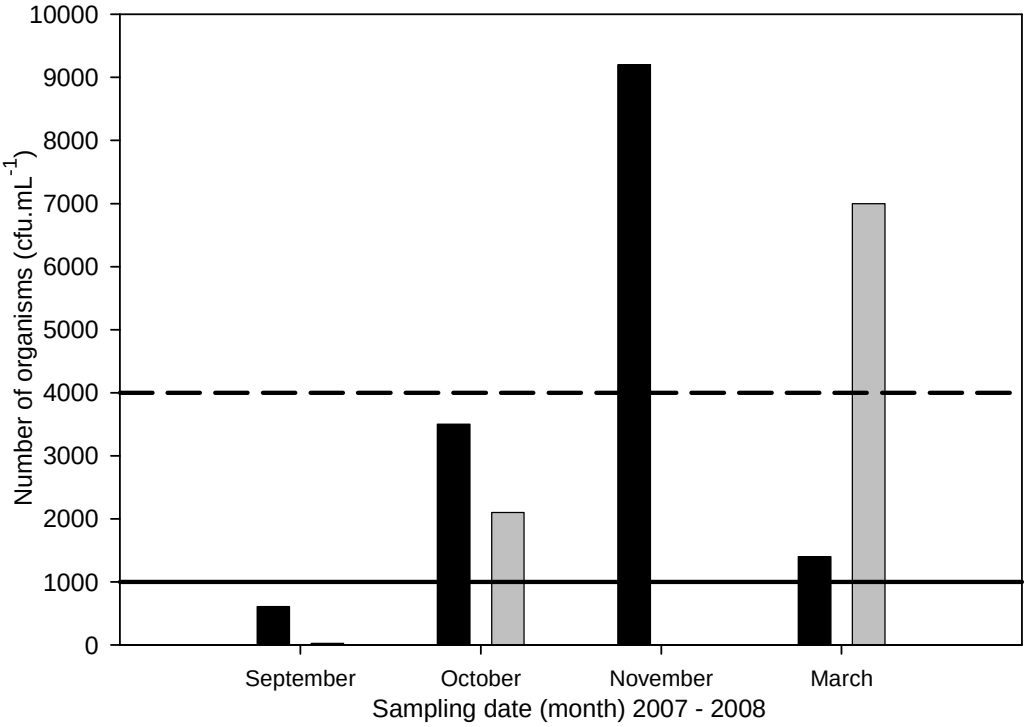

Figure 4 Compliance of river water from Plank 3 site with the South African guidelines (DWAF, 1996a; DWAF, 2008), in terms of coliform (black) and faecal coliform (grey) counts in water used for recreational purposes or the irrigation of foods eaten raw or minimally processed (Dotted line = DWAF guideline; solid line = WHO guideline (WHO, 1989)).

At Plank 2, faecal coliform counts peaked at 54 000 org.100 mL^{-1} during October 2007 (Fig. 3). Although levels of faecal coliforms were generally lower than those found at Plank 1, they still did not comply with the guidelines for irrigation water (DWAF, 1996a). The lower microbial counts seen here could possibly be attributed to the natural die-off of these organisms, the fact that this sampling point was situated further away from the sources of contamination, and dilution of the contamination after the confluence with the less polluted Eerste River.

The faecal coliform counts found at Plank 3 (Fig. 4) were much lower than those seen at the other two sites in this river. During September 2007, when counts of 23 org.100 mL^{-1} were noted, this water was suitable for the irrigation of fruits and grapes providing that the fruits were not wetted. This water would also be suited for the irrigation of any crop not to be eaten raw by means of any irrigation method (DWAF, 1996a). During November 2007, when no faecal coliforms could be detected, this water was suitable for the irrigation of any crop by means of any irrigation method, with very little likelihood for

the transmission of disease. Apart from the natural die-off of the organisms and the fact that this site is situated furthest from the sources of contamination, possible explanations for the low faecal coliform counts include the following: dilution of the contamination after the confluence of the Eerste River with the Plankenburg River at Plank 2; and the greater exposure of organisms to environmental factors (eg. UV radiation from the sun) in the shallower channel.

At Mossel 4, faecal coliform counts did not once over the sampling period comply with the South African quality guidelines for irrigation water (DWAF, 1996a) and peaked at 460 000 org.100 mL^{-1} in November 2007 (Fig. 5). It was evident that this water was severely contaminated and definitely not suited for the irrigation of the specific farmer's vegetables, some of which were to be eaten raw or minimally processed.

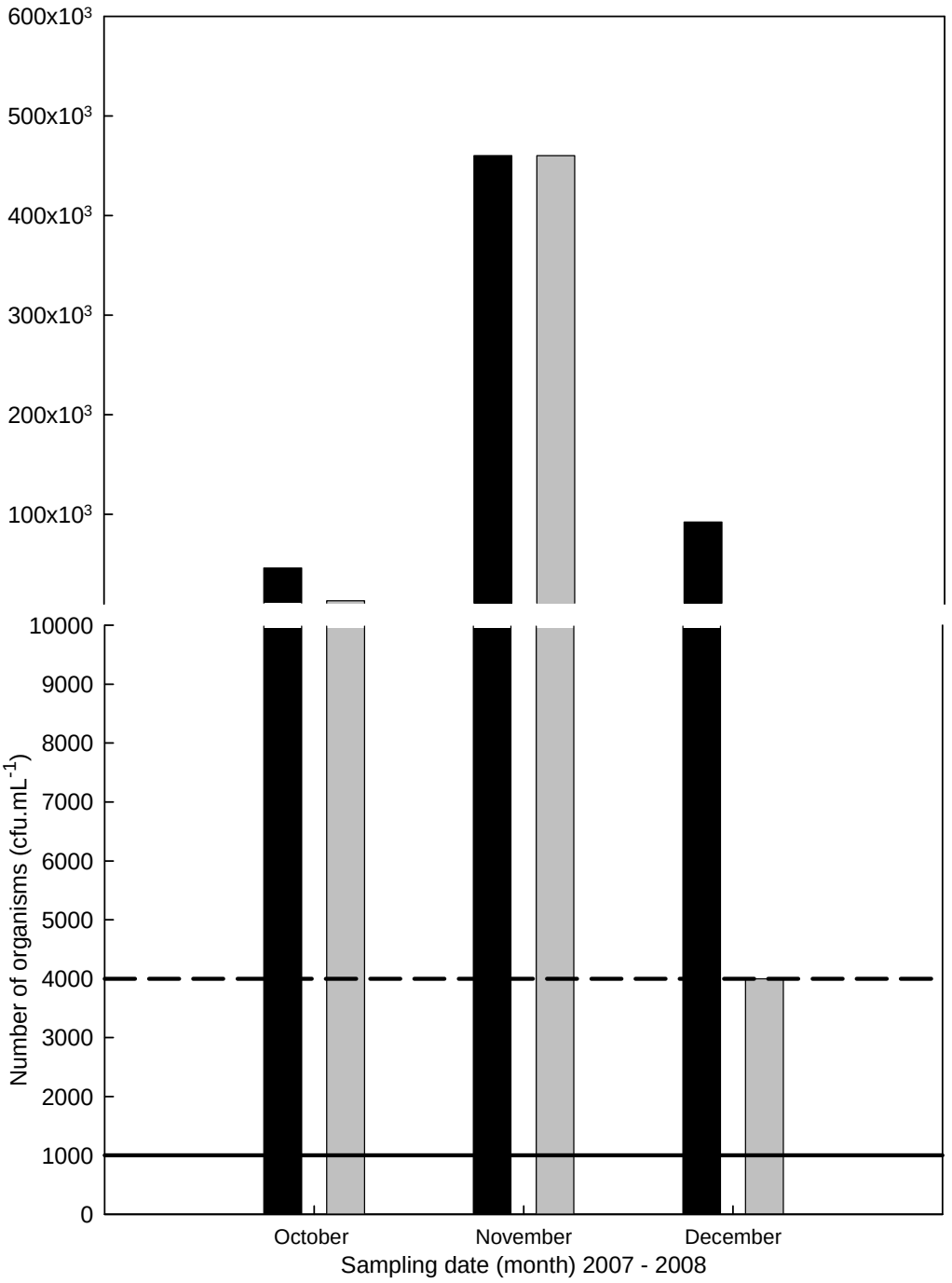

Figure 5 Compliance of river water from Mossel 4 site with the South African guidelines (DWAF, 1996b; DWAF, 2008), in terms of coliform (black) and faecal coliform (grey) counts in water used for the irrigation of foods eaten raw or minimally processed (Dotted line = DWAF guideline; solid line = WHO guideline (WHO, 1989)).

Species identification of organisms isolated from river and irrigation water – After isolating the colonies using the methods as described, those exhibiting the typical morphology as described for each organism were purified. A Gram stain was done; the stains were microscopically examined and all observations noted. As a final measure, catalase and oxidase tests were performed before an API identity best suited for the unidentified organism was chosen. The API was inoculated and incubated as set-out in the instruction manual, and the organism identified making use of the API web software.

Typical colony characteristics (Figs. 6 and 7), as described by Merck (2005), were often noted but colonies exhibiting other morphological properties, not typical to that of the organisms in question, were also found. These colonies were also identified as described above. Organisms isolated from the rivers included:

- *Escherichia coli*
- *Enterobacter aerogenes*
- *Enterobacter cloacae*
- *Proteus mirabilis*
- *Listeria grayii*
- *Klebsiella pneumoniae subsp. pneumoniae*

Figure 6 Typical colonies of *E. aerogenes* (left) and *E. coli* (right) on L-EMB agar.

Figure 7 Typical colonies of *E. cloacae* (left), *E. aerogenes* (middle) and *K. pneumoniae subsp. pneumoniae* (right) on XLD agar.

Mosselbank River, irrigation water and irrigated produce data

As it was evident in the baseline river study that the water from the Mosselbank river contained high levels of faecal contamination, it was decided to continue monitoring this river, the irrigation water and some irrigated produce with the consent of the farmer. This site was thus further monitored every three weeks for a period of eight months.

Chemical analysis – The chemical analyses conducted on the river and irrigation water samples are summarised in Table 3. Again, a slight relationship between water temperature and pH value was noted as the pH decreased from 6.53 to 6.20 as the temperature decreased from 23.7℃ in February to 14 .7℃ in September. The pH values of the river water was within the TWQR of the South African quality guidelines for irrigation water (DWAF, 1996a) once, and although this water could still be suitable for the irrigation of crops, the possible detrimental effects this could have on crop yield and irrigation equipment should be considered (DWAF, 1996a). No relationship between either of the abovementioned parameters and the COD, alkalinity or conductivity of the samples could be seen. The alkalinity ranged between 60 and 150 mg $CaCO_3.L^{-1}$, while the conductivity of the samples ranged between 60 and 77 $mS.m^{-1}$. COD values of between 35 and 63 $mg.L^{-1}$ were noted during the sampling period.

The pH of the irrigation water, taken at the point of irrigation, ranged between 7.03 and 8.10 throughout the sampling period, staying relatively stable from May to September. These values fell within the TWQR for the pH of irrigation water, and would have no detrimental effects on crop yield, soil quality or irrigation equipment (DWAF, 1996a). Again, no relationship between the pH and the COD, alkalinity or conductivity was seen. The alkalinity ranged between 100 and 175 mg $CaCO_3.L^{-1}$, while the conductivity of the samples ranged between 50 and 106 $mS.m^{-1}$. COD values of between 25 and 50 $mg.L^{-1}$ were noted during the sampling period

Table 3 Chemical analysis of water from the Mosselbank River.

Sampling date	Temperature (℃)	pH	Alkalinity (mg $CaCO_3.L^{-1}$)	Conductivity ($mS.m^{-1}$)	COD ($mg.L^{-1}$)
River					
Feb 2008	23.7	6.53	100	70	63
Mar 2008	25.4	6.82	125	68	50
Apr 2008	22.8	6.38	125	70	45
May 2008-a	16.5	6.07	150	70	50
May 2008-b	17.1	6.10	120	60	50
Jun 2008	15.4	6.10	120	60	45
Jul 2008	16.1	6.15	80	70	50
Aug 2008	16.3	6.12	100	77	35
Sept 2008	14.7	6.20	60	60	50
Irrigation					
Mar 2008	-	8.10	175	106	50
Apr 2008	-	7.43	175	93	37
May 2008-a	-	7.08	125	80	45
May 2008-b	-	7.20	175	70	41
Jun 2008	-	7.06	120	70	50
Jul 2008	-	7.17	125	60	45
Aug 2008	-	7.03	120	70	40
Sept 2008	-	7.20	100	50	25

Microbial enumeration – After microbiological tests were done on the river water, irrigation water and vegetable samples, the following bacterial data, as given in Tables 4 and 5, were obtained.

During February 2008 ACC levels of 12 800 000 000 $cfu.mL^{-1}$ were found in the river sample. On this occasion an irrigation water sample could unfortunately not be taken due to bad weather, but the corresponding ACC counts on the two irrigated lettuce samples were noted as being 11 900 000 $cfu.mL^{-1}$ and 9 700 000 000 $cfu.mL^{-1}$, respectively. The South African guidelines for water for domestic use states that more than 1 000 $cfu.mL^{-1}$

could be seen to having an increased risk for the transmission of disease (DWAF, 1996c). Although this water was used for irrigation purposes, it was applied to foods intended to be eaten raw or minimally processed, and the high levels of contamination found on the irrigated lettuce reflected that this water is best not used for irrigation. Aerobic and anaerobic sporeformer counts of 130 cfu.mL^{-1} and none detected, respectively, were found in the river water, while 110 cfu.mL^{-1} aerobic and 350 cfu.mL^{-1} anaerobic sporeformers were noted for the lettuce samples. Coliform and faecal coliform counts of 5 400 org.100 ml^{-1} were found in the river water. These do not fall within the TWQR for irrigation water which is set at less than 1 000 org.100 mL^{-1}. The wash water from the irrigated lettuce contained quantities of 3 500 and 1 600 000 org.100 mL^{-1}, respectively. According to the guidelines used for the microbial quality of raw vegetables and raw fruits (DoH, 2006), these samples should not contain more than 200 coliform bacteria per gram and zero *E. coli* organisms should be present per gram of food. Apart from this, typical colonies of *E. coli*, *Salmonella* and *Listeria* were isolated from the water and both of the lettuce samples. The Department of Health guidelines (DoH, 2006) stipulates that *Salmonella spp*. be absent in 25 g of sample, while *Listeria spp*. should be absent in 1 g of sample. As both of these organisms were present, this produce could be seen as unfit for human consumption.

River water samples taken during March had ACC levels of 61 000 cfu.mL^{-1} (Table 5), while the irrigation water and two lettuce samples had counts of 20 500, 460 000 and 228 000 cfu.mL^{-1}, respectively. The higher counts found on the lettuce samples indicated the possible accumulation of contamination on the samples by means of repeated irrigation. Aerobic sporeformers in the river peaked at 9 000 cfu.mL^{-1}, but were much lower in the other samples taken on this date. Anaerobic sporeformers were not detected for the irrigation water and one of the lettuce samples, while counts of 30 cfu.mL^{-1} were found on the other lettuce sample and in the river water. Although coliform counts of 1 600, 70, 350, and 130 org.100 mL^{-1} were noted for the river, irrigation water and two lettuce samples, respectively, no faecal coliforms, *E. coli* or Staphylococci were found in or on any of the samples. This means that this water would be suitable for the irrigation of crops intended to be eaten raw or minimally processed (DWAF, 1996a). However, typical colonies of *Salmonella* and *Listeria* were isolated from all of the samples taken on this date. This again questions the accuracy of using faecal coliforms and *E. coli* as indicators for the presence of other human pathogens.

Table 4 Microbial loads present in water taken from the Mosselbank River, in water taken from the irrigation dam, and on the irrigated lettuce.

Date	Point	ACC (cfu.mL^{-1})	Aerobic spores (cfu.mL^{-1})	Anaerobic spores (cfu.mL^{-1})	Coliforms (org.100mL^{-1})	Faecal (org.100mL^{-1})	*E. coli*	*Salmonella*	*Listeria*	*Staph* (cfu.mL^{-1})	*Enterococci* (cfu.100mL^{-1})
Feb 2008	River	12 800 000 000	130	ND	5400	5400	TG	TG	TG	140	38
	Lettuce	11 900 000	110	350	3500	3500	TG	TG	TG	ND	ND
	Lettuce	9 700 000 000	90	ND	1600000	1600000	TG	TG	TG	200	ND
Mar 2008	River	61000	9000	30	1600	ND	ND	TG	TG	ND	37
	Irrigation	20500	170	ND	70	ND	ND	TG	TG	ND	16
	Lettuce	460000	80	30	350	ND	ND	TG	TG	ND	72
	Lettuce	228000	20	ND	130	ND	ND	TG	TG	ND	55
Apr 2008	River	830000	1185	150	1600000	1600000	TG	TG	TG	4600	ND
	Irrigation	4100	50	ND	ND	ND	ND	ND	ND	ND	ND
	Lettuce	420000	30	ND	23	ND	ND	TG	TG	2300	ND
	Lettuce	244000	10	60	23	4.5	TG	TG	TG	120	ND
May 2008	River	32200	1000	670	160000	35000	TG	TG	TG	110	ND
	Irrigation	6500	1000	20	350	33	TG	TG	TG	300	ND
	Lettuce	57000	1000	1900	4.5	4.5	TG	TG	TG	500	ND
	Lettuce	410000	3000	2600	540000	330	TG	TG	TG	2000	ND

ND = None detected;*TG = Typical growth, ACC = Aerobic Colony Count.

Table 5 Microbial loads present in water taken from the Mosselbank River, in water from the irrigation dam, and on the irrigated cabbage.

Date	Point	ACC (cfu.mL^{-1})	Aerobic spores (cfu.mL^{-1})	Anaerobic spores (cfu.mL^{-1})	Coliforms (org.100mL^{-1})	Faecal (org.100mL^{-1})	*E. coli*	*Salmonella*	*Listeria*	*Staph* (cfu.mL^{-1})	*Enterococci* (cfu.100mL^{-1})
May 2008	River	770000	ND	ND	17000	3500	ND	TG	ND	6000	ND
	Irrigation	12400	10	ND	540	49	ND	TG	ND	ND	ND
	Cabbage	1330000	30	ND	49	1.8	ND	TG	ND	5600	ND
	Cabbage	1580000	ND	ND	49	1.8	ND	TG	TG	1070	ND
Jun 2008	River	88000	290	ND	3500	1700	TG	TG	ND	60	ND
	Irrigation	11300	30	10	220	7.8	TG	TG	ND	80	ND
	Cabbage	75000	ND	ND	540	2	ND	TG	ND	110	ND
	Cabbage	166000	10	ND	33	ND	ND	TG	TG	250	ND
Jul 2008	River	24600	400	30	1100	540	TG	TG	TG	50	ND
	Irrigation	34000	700	200	1100	32	TG	TG	ND	75	ND
	Cabbage	3600000	1000	20	1600000	ND	ND	TG	TG	80	ND
	Cabbage	5300000	100	ND	350000	1600	TG	TG	TG	50	ND
Aug 2008	River	12 400	500	200	3500	440	TG	TG	TG	60	ND
	Irrigation	3300	ND	ND	34	7.8	TG	TG	ND	30	ND
Sept 2008	River	35000	ND	50	330000	4900	TG	TG	TG	950	ND
	Irrigation	43000	5100	ND	490000	490000	TG	TG	TG	280	ND
	Cabbage	1910000	3200	120	7900	ND	ND	TG	TG	96000	ND
	Cabbage	680000	1000	190	2200	7.8	TG	TG	ND	520	ND

During April, ACC levels of 830 000 cfu.mL^{-1} were noted for the river water (Table 5), while levels of 4 100, 420 000 and 244 000 cfu.mL^{-1} were found in the irrigation water and two lettuce samples, respectively. Although higher amounts of aerobic sporeformers (1 185 cfu.mL^{-1}) were found in the river water, low numbers of these organisms and the anaerobic sporeformers were found in the rest of the samples. Coliforms and faecal coliforms in the river were noted as 1 600 000 org.100 mL^{-1}, while none of these organisms were found in the irrigation water and very low numbers were found on the lettuce samples. Even with counts as low as 4 faecal coliforms per 100 mL of wash water, typical colonies of *E. coli*, *Salmonella* and *Listeria* were isolated from at least one of the lettuce samples. No Staphylococcus spp. were isolated from the irrigation water, but levels of 4 600, 2 300 and 120 cfu.mL^{-1} were found in the river water and on the two lettuce samples, respectively. No faecal enterococci were detected in any of the samples taken on this day.

On the 5th of May the last of the lettuce samples were taken. For this set of samples the ACC levels were found to be: 32 200 cfu.mL^{-1} in the river water; 6 500 cfu.mL^{-1} in the irrigation water; and 57 000 and 410 000 cfu.mL^{-1} for the two lettuce samples, respectively. Higher than usual counts of aerobic and anaerobic sporeformers were noted for most of the samples. Again, higher levels of coliform bacteria and faecal coliforms were present in the river water and on the lettuce samples than in the irrigation water, suggesting die-off of these organisms in the irrigation dam and during irrigation, but clearly a subsequent build-up of contamination on the lettuce as a result of the multiple applications of the irrigation water. Typical colonies of *E. coli*, *Salmonella* and *Listeria* were isolated from all of the samples taken, suggesting that this water and produce may pose a risk to the health of consumers. Although no faecal enterococci were detected, Staphylococci levels of as high as 2 000 cfu.mL^{-1} were found on one of the lettuce samples. This concluded the sampling of the lettuce, and from here forth cabbage heads were sampled for the duration of the study.

For the first samples taken in May 2008 (May 2008-a), an ACC level of 770 000 cfu.mL^{-1} was found for the river water while the irrigation water contained 12 400 cfu.mL^{-1} (Table 5). The corresponding ACC counts on the two irrigated cabbage samples were 1 330 000 and 1 580 000 cfu.mL^{-1}, respectively. The South African guidelines for water for domestic use state that more than 1 000 heterotrophic bacteria per millilitre could be seen as to have an increased risk for the transmission of disease (DWAF, 1996c). Although this water was used for irrigation purposes, it was applied to produce that could be eaten raw or after a minimal processing step. The high levels of contamination found on the irrigated

crops indicate that this water might not be suited for irrigation purposes. Aerobic sporeformer counts of 10 cfu.mL^{-1} were found in the irrigation water and 30 cfu.mL^{-1} were detected on one of the cabbage samples. No aerobic sporeformers were detected on the other two samples, while no anaerobic sporeformers were detected in any of the samples. Coliform and faecal coliform counts of 17 000 and 3 500 org.100 mL^{-1} were found in the river water. These do, once again, not fall within the TWQR for irrigation water. The irrigated cabbage samples both had 49 org.100mL^{-1} coliforms and 1 org.100 mL^{-1} faecal coliforms, while the irrigation water contained 49 faecal coliforms per 100 mL water. No *E. coli* colonies were isolated from any of the samples, but typical growth of *Salmonella* was seen for all of the samples and growth of *Listeria* was seen on one of the cabbage samples. As both of these organisms were present, this produce could be seen as unfit for human consumption (DoH, 2006).

The river water sample (Table 5) taken in June had an ACC level of 88 000 cfu.mL^{-1}, while the irrigation water and two cabbage samples had counts of 11 300, 75 000 and 166 000 cfu.mL^{-1}, respectively. Again, the higher counts found on the cabbage samples could indicate a build-up of contamination with repeated irrigation. The aerobic sporeformer count in the river was noted to be 290 cfu.mL^{-1}, while very low counts were found on the other samples. Anaerobic sporeformers were absent on most of the samples taken on this date. Coliform bacteria present in the samples were 3 500 org.100mL^{-1} in the river water, 220 org.100 mL^{-1} in the irrigation water, and 540 and 33 org.100 mL^{-1} on each of the two cabbage samples. The faecal coliform level in the river was 1 700 org.100 mL^{-1}, while faecal organisms were found in the irrigation water and on the irrigated cabbages. A possible explanation for the lower counts found on these samples could be that during the winter rains the need for the irrigation of the produce decreases and rain water could “wash” some of the contamination off the crops. Apart from this, the lower ambient temperatures during the winter season may hinder the growth of these organisms. Nonetheless, typical colonies of *Listeria* and *Salmonella* were found on the cabbages, while typical colonies of *E. coli* and *Salmonella* were isolated from the river and irrigation water samples. No faecal enterococci were isolated from any of the samples taken between May and September.

During July 2008 (Table 5), ACC levels of 24 600 cfu.mL^{-1} were noted for the river water, while levels of 34 000, 3 600 000 and 5 300 000 cfu.mL^{-1} were found in the irrigation water and lettuce samples, respectively. Both the river and the irrigation water samples had counts of 1 100 org.mL^{-1} coliform bacteria, while those for the cabbage samples were 1 600 000 and 350 000 org.100 mL^{-1}, respectively. The faecal coliform

count in the river water was 540 org.100 mL^{-1}, while levels of 32 org.100 mL^{-1} and 1 600 org.100 mL^{-1} were noted for the irrigation water and one cabbage sample, respectively. On the other cabbage, no faecal coliforms were detected. *Listeria* was not detected in the irrigation water sampled on this date, but typical colonies of *E. coli*, *Salmonella* and *Listeria* were isolated from all of the other samples. Levels of 50, 75, 80 and 50 cfu.mL^{-1} were found in the river water, irrigation water and the two cabbage samples, respectively.

During August 2008 (Table 5), no cabbages could be sampled due to very bad weather. The microbial loads of the two water samples taken on this occasion were very low. This could be due to the heavy rains received in the week prior to the date of sampling. Although no *Listeria spp*. was detected in the irrigation water, typical colonies of *E. coli*, *Salmonella* and *Listeria* were isolated from the irrigation sample.

On the last sampling occasion (Table5), ACC counts of 35 000, 43 000, 680 000 and 1 910 000 cfu.mL^{-1} were noted for the river water, irrigation water and each of the cabbages, respectively. Higher than usual levels of aerobic sporeformers were found in the irrigation water and on the cabbage samples, while none of these organisms were detected in the river water. From all the samples taken during the winter months, the highest counts of coliform and faecal coliform bacteria were found in the river and irrigation waters sampled on this date. The counts of these organisms found on the irrigated produce were much lower than in the water samples. This was probably as a result of the fewer applications of irrigation water during the rainy months. Still, *E. coli* and *Listeria* colonies were isolated from all but one of the cabbage samples, while the growth of *Salmonella* was present for all of the samples. This, once again, shows the limitations of using faecal coliforms as an indicator of the presence of other human pathogens. The highest amount of Staphylococcus found on any of the samples (96 000 cfu.mL^{-1}) was found on the cabbage sample where no faecal coliforms were detected.

Over the period of February to September 2008, the river water only complied with the guidelines (DWAF, 2008) five times. This guideline is set at 4 000 faecal coliforms per 100 mL of water when irrigating crops to be eaten raw or minimally processed. The water taken from the irrigation dam complied with these guidelines seven of the eight times it was sampled. The lettuce heads sampled between February and May 2008 complied with the guidelines (DoH, 2006) three out of the eight times sampled, while the cabbages sampled between May and September 2008, complied with the same guidelines six times. According to these guidelines, no E. coli should be present in a 25 g sample of raw vegetables or fruit (DoH, 2006). Apart from this, *Listeria* and *Salmonella* species were

isolated from these vegetables on several occasions, making this a definite health risk to those handling and consuming this produce.

Species identification of organisms isolated from the river water, irrigation water and vegetable samples - After a pure culture of the colonies exhibiting typical morphology was obtained by the methods described earlier, a Gram-stain, oxidase and catalase test were done to determine which API test strip would be most suitable for identifying the isolated organism. The selected API strips were inoculated and incubated as indicated in the instruction manual, before the API web software was used to identify the organism. This was done to determine whether the same organisms could be isolated from the river water, irrigation water and the lettuce samples, indicating a possible carry-over of microbes from the irrigation water to the irrigated produce. The following organisms were isolated from the river, irrigation water and produce samples taken from the Mosselbank site. A detailed description of where each organism was found will be given in a later section entitled “possible links”.

- *Citrobacter freundii*
- *Enterobacter aerogenes*
- *Enterobacter cloacae*
- *Eschericia coli*
- *Klebsiella pneumonia*
- *Listeria grayi*
- *Listeria ivanovii*
- *Listeria monocytogenes*
- *Listeria seeligeri*
- *Listeria sp.*
- *Proteus mirabilis*
- *Salmonella spp*.
- *Staphylococcus aureus*
- *Staphylococcus saprophyticus*

Microbial loads on vegetables from local fresh produce markets

To see how the microbial loads present on the samples irrigated with contaminated water compared with the loads on produce irrigated with water that is considered to be of good quality, cabbage and lettuce samples were taken from a farm where the products are irrigated with water from the Theewaterskloof dam. Cabbage and lettuce samples were also purchased at a local fresh produce market to assess how the “contaminated” produce compared to those found on the retail market (Table 6).

Theewaterskloof produce - The aerobic colony counts of the produce irrigated with water from the Theewaterskloof dam was on average lower than that found on the Mosselbank products (Table 6), while the levels of aerobic and anaerobic spore formers present on the products were more or less equal. Although the total coliform and faecal coliform counts

of the Theewaterskloof products were much lower than that found on the Mosselbank produce, it still did not comply with the guidelines of the Department of Health (DoH, 2006). Although typical growth of *Salmonella* was not found, typical colonies of *E. coli* and *Listeria* was present. Enterococci were not detected on any of the products, but Staphylococci counts exceeding 100 cfu.mL^{-1}, were found.

Local fresh market produce - The cabbage from the fresh produce market had an aerobic colony count of 1 090 000 cfu.mL^{-1}, much the same as that found on the Mosselbank cabbage samples, while lower levels of the spore formers were found on both the market products (Table 6). Again, low levels of total and faecal coliforms were found to be present on the products bought at the market, but they still did not comply with the standard of zero faecal coliforms present per one gram of sample tested (DoH, 2006). Typical growth of *Listeria*, *Salmonella* and *E. coli* was found, suggesting that these products could pose a possible threat to the health of the food handlers and consumers. Low levels of Staphylococci were found while intestinal enterococci were not detected on either of the products.

Although the levels of microbial contaminants found on these products were lower than that of the Mosselbank products, typical growth of possible human pathogens were present and therefore a possible risk of infection existed.

Species identification of organisms found on the Theewaterskloof and Fresh Produce Market samples – As with the previous colonies exhibiting typical growth, the necessary biochemical tests were done and the organisms were identified with the use of the API web software. These organisms included:

- *Enterobacter aerogenes*
- *Escherichia coli*
- *Listeria grayii*
- *Listeria inocua*

POSSIBLE LINKS

To determine whether a link exists between the contamination in the river water and that found on the produce, the organisms isolated and identified from each of the different samples were tabulated (Table 7).

Table 6 Microbial loads present on products irrigated with water considered to be of good quality and products available on the retail market.

Date	**Point**	**ACC** (cfu.mL^{-1})	**Aerobic spores** (cfu.mL^{-1})	**Anaerobic spores** (cfu.mL^{-1})	**Coliforms** (org.100mL^{-1})	**Faecal** (org.100mL^{-1})	***E. coli***	***Salmonella***	***Listeria***	***Staph*** (cfu.mL^{-1})	***Enterococci*** (cfu.100mL^{-1})
Aug 2008	Thee – L	660 000	100	100	49	17	TG	ND	ND	180	ND
Aug 2008	Thee – C	161 000	300	40	17	7.8	TG	ND	TG	110	ND
Aug 2008	Mrkt - L	279 000	20	ND	79	49	TG	TG	TG	30	ND
Aug 2008	Mrkt - C	1 090 000	50	20	11	4.5	TG	ND	ND	50	ND

Thee – X = Theewaterskloof lettuce (L) or cabbage (C); Mrkt – X = Market lettuce (L) or cabbage (C).

Table 7 Organisms isolated from the river water, irrigation water and irrigated products.

Organism	River water	Irrigation water	Lettuce	Cabbage
C. freundii	X		X	
E. aerogenes	X	X	X	X
E. cloacae	X	X	X	X
E. coli	X	X	X	X
Listeria sp.	X	X	X	X
L. grayii	X		X	X
L. ivanovii	X			
L. monocytogenes	X			
L. seeligeri	X		X	X
P. mirabilis	X	X		X
Staphylococcus sp.	X	X	X	X
S. aureus	X			X
S. saprophyticus			X	
Salmonella sp.	X	X		

With the exception of *S. saprophyticus*, all the organisms listed in Table 7 were present in the river water. *E. aerogenes*, *E. cloacae*, *E. coli* and *P. mirabilis* were isolated from the river water, irrigation water and at least one of the lettuce and cabbage samples, suggesting a carry-over of some of the organisms onto the produce. *Listeria monocytogenes* was found in the river water but not in any of the other samples, but this in itself is alarming as this organism poses a serious threat to the health of consumers – especially pregnant mothers! *Staphylococcus. saprophyticus* was found on the lettuce samples but not in any of the other samples. This could be an indication of another source of contamination but it is also possible that, since the river and irrigation waters were sampled only once every three weeks, the presence of the organism in the water could have been "missed" (low numbers, low sensitivity of methods) but it survived long enough on the cabbage samples for it to be isolated.

CONCLUSIONS AND RECOMMENDATIONS

When looking at the data discussed above, it is evident that the water of the Plankenburg River is heavily contaminated and does not comply with the DWAF guidelines for water for the irrigation of foods to be eaten raw or minimally processed. The faecal coliform counts

at site Plank 1 in the Plankenburg River were higher than the counts at site Plank 3. Apart from the influence the natural decay of these organisms may have on the levels, an explanation for the higher counts may be that site one is located much closer to the probable source of contamination – the partially unserviced Kayamandi Informal Settlement. Since potential human pathogens were isolated from this river during this study, it can be concluded that the water might pose a threat to the health of individuals coming into contact with it. It is thus recommended that the appropriate authorities are made aware of this problem and that the sanitary services in Kayamandi receive urgent attention.

The study also showed high levels of faecal coliforms and other pathogens in the waters of the Mosselbank River, and thus this water is not suitable for the irrigation of minimally processed foods. In this case the "treated" water discharged from the Kraaifontein Sewage Works might be the source of contamination. Even though lower levels of microbial contaminants were found in the irrigation water, cases of non-compliance were still found and some potential pathogens were isolated from these samples. As for the river water, the irrigation water can also be seen as a possible source of infection to farm workers and others coming into contact with it.

The faecal coliform levels found on the products exceeded that of the irrigation water in most cases. Apart from the possible environmental contaminants such as the defecation of birds, a "build-up" of organisms from repeated irrigation may be the explanation for this. Several potential pathogens such as *E. coli*, *E. aerogenes* and *E.cloacae* were isolated from the lettuce and cabbage samples and the faecal coliform counts were found to exceed the guidelines of the Department of Health (DoH, 2006). It is evident that these products could pose a risk of infection to the farm workers handling the produce and the consumers ingesting these products without further processing.

The Theewaterskloof and market lettuce and cabbage samples had lower levels of contaminants, but pathogens including *E. coli* were still found to be present. These products are available on the retail market and are bought by many non-suspecting consumers. These retailers should require the farmers to give proof that their products are safe for consumption and comply with all the regulations of the involved authorities, and farmers, in turn, should demand that DWAF take action against the pollution of the country's freshwater resources.

On numerous occasions, the same pathogens were isolated from the river water, irrigation water and the irrigated produce – evidence that contaminated irrigation water can indeed carry pathogens over onto irrigated products. To be absolutely sure that these

organisms are indeed of the same origin, future researchers should make use of molecular methods such as DNA sequencing for identification and comparison of the different organisms.

The methods used during this study are internationally recommended methods used in the food industry and are also standard methods for the investigation of water quality. During the study however, several colonies unlike any of those typically described in the methods were found. Many of these were identified as organisms with different nutrient requirements than that of the organism in question. It is thus recommended that future research be done on the selectivity of the recommended media and the fact that possible mutation of organisms to survive in their "new" habitat outside the digestive tract of humans or other warm-blooded animals is investigated. This could also be the reason why some isolates were not identifiable using the API system. The impact that the presence of potential pathogens could have on the entire food industry should also be considered. It is also possible that, based on the standard methods, some products are rejected while there is actually no reason to do so, while others are approved for retail while they could contain serious human pathogens.

After finding that the rivers in question are indeed contaminated and that their use for irrigation of fruits and vegetables intended to be eaten raw, it is clear that these products may be a serious threat for the foodborne infection of consumers. Most of the Western Cape's fresh produce is intended for export, but if these products are considered a threat to consumer health, they will no longer be accepted for export leaving many farmers and farm workers without a source of income and the province in a serious economical predicament.

REFERENCES

Allende, A., McEvoy, J., Tao, Y. & Luo, Y. (2009). Antimicrobial effect of acidified sodium chlorite, sodium chlorite, sodium hypochlorite, and citric acid on *Escherichia coli* O157:H7 and natural microflora of fresh-cut cilantro. *Food Control*, **20**, 230–234.

APHA (American Public Health Association). (1998). *Standard Methods for the Analysis of Water and Wastewater*. New York Academic Press: New York

Barnes, J.M. (2007). Department of Community Health, Faculty of Health Sciences, University of Stellenbosch, Stellenbosch, South Africa. Personal communication.

Barnes, J. M. (2008). Department of Community Health, Faculty of Health Sciences, University of Stellenbosch, Stellenbosch, South Africa. Personal communication.

Beuchat, L. R. (1996). Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection*, **59**, 204–216.

Britz, T.J., Barnes, J.M., Buys, E.M., Ijabadeniyi, O.A., Minnaar, M., Potgieter, N., Sigge, G.O., Ackermann, A., Lötter, M., Taylor, M.B., van Zyl, W., Venter, I. & Netshikweta, R. (2007). Quantitative investigation into the link between irrigation water quality and food safety. *Water Research Commission Project Report K5/1773//4*, Pretoria, South Africa.

DoH (Department of Health) (2006). Guidelines for environmental health officers on the interpretation of microbiological analysis data of food. Department of Health, directorate: Food Control. Annexure B: Proposed microbiological specification to be used as guidelines for foods. Government Printers: Pretoria.

DWAF (Department of Water Affairs and Forestry). 1996a. South African Water Quality Guidelines (second edition). Volume 4: Agricultural Water Use: Irrigation. CSIR Environmental Services: Pretoria.

DWAF (Department of Water Affairs and Forestry). 1996b. South African Water Quality Guidelines (second edition). Volume 2: Recreational Water Use. CSIR Environmental Services: Pretoria.

DWAF (Department of Water Affairs and Forestry). 1996c. South African Water Quality Guidelines (second edition). Volume 1: Domestic Use. CSIR Environmental Services: Pretoria.

DWAF (Department of WaterAffairs and Forestry) (2008). Guidelines for assessing the potential health risk for the four water uses. National Microbial Monitoring Programme. Bellville, South Africa.

Greig, J.D. & Ravel, A. (2009). Analysis of foodborne outbreak data reported internationally for source attribution. *International Journal of Food Microbiology*, **130**, 77 – 87.

ISO 4833 (1991). Microbiology: General Guidance for the enumeration of micro-organisms – Colony count technique at 30ºC. SABS Printers: Pretoria.

Map 3318 DD (2003). Map of Stellenbosch Surroundings. Published by the Chief Directorate: Surveys and Mapping, Mowbray, Cape Town.

Merck. (2005). Merck Microbiology Manual, 12th edition. Merck: KGaA, Darmstadt, Germany.

MFHPB 19 (2002). Health Products and Food Branch, Ottawa: Enumeration of Coliforms, Faecal Coliforms and of *E. coli* in foods using the MPN method. Canadian Department of Health: Ottawa.

MFLP 44 (1998). Health Protection Branch Ottawa: Determination of Aerobic and Anaerobic Sporeformers. Canadian Department of Health: Ottawa.

SABS 11290-1 (2004). Microbiology of food and animal feeding stuffs: Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 1: Detection method. SABS Printers: Pretoria.

SABS 6579 (2004). Microbiology: General guidance on the methods for the detection of *Salmonella*. SABS Printers: Pretoria.

SABS 6888-1 (1999). Microbiology of Food and Animal Feeding stuffs: Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1: Technique using Baird-Parker agar medium. SABS Printers: Pretoria.

SANS 5667-6 (2006). Water quality – Sampling, Part 6: Guidance on sampling of rivers and streams. SABS Printers: Pretoria.

SANS 7899-2 (2004). Water quality: Detection and enumeration of intestinal enterococci, membrane filter method. SABS Printers: Pretoria.

Taylor, M.B. (2007). Department of Medical Virology, University of Pretoria, Pretoria, South Africa. Personal Communication.

Taylor, M.B. (2008). Department of Medical Virology, University of Pretoria, Pretoria, South Africa. Personal Communication.

Velazquez, L.C., Barbini, N.B., Escudero, M.E., Estrada, C.L. & de Guzman, A.M.S. (2009). Evaluation of chlorine, benzalkonium chloride and lactic acid as sanitizers for reducing *Escherichia coli* O157:H7 and *Yersinia enterocolitica* on fresh vegetables. *Food Control*, **20**, 262–268.

Wadhwa, S.G., Khaled, G.H. & Edberg, S.C. (2002). Comparative Microbial Character of Consumed Food and Drinking Water. *Critical Reviews in Microbiology*, **28** (3), 249-279.

WHO (World Health Organisation). (1989). Health guidelines for the use of wastewater in agriculture and aquaculture. Geneva, Switzerland: World Health Organisation. Technical Report Series 778.

Yuk, H-Y., Yoo, M-Y., Yoon, J.W., Moon, K.D., Marshall, D.L. & Oh, D.H. (2006). Effect of Combined ozone and organic acid treatment for control of Escherichia coli O157:H7 and Listeria monocytogenes on lettuce. *Journal of Food Science*, **71** (3), 83–87.

CHAPTER 4
GENERAL DISCUSSION AND CONCLUSIONS

Agriculture is one of the key economic sectors in the Western Cape and provides many job opportunities (WESGRO, 2006). Annual exports already exceed R 7 billion (WESGRO, 2006) and this is bound to increase as the demand for fresh fruit and vegetables by health-conscious consumers increases (Beuchat, 1996; NWRS, 2004). South Africa is a semi-arid country and therefore the success of the agricultural industry depends greatly on the use of irrigation water from rivers and dams (NWRS, 2004).

The Western Cape's employment sector cannot keep up with the increased rate of urbanisation and population growth, and therefore, many families are left destitute and are forced to seek refuge in informal settlements (Stats SA, 2006). As many of these settlements have no basic sanitation infrastructure (DEAT, 2006), pressure is put on the nearby water sources to supply in the domestic needs of all these families. The total deficit of sanitary infrastructure in these areas makes the faecal contamination of these water sources unavoidable (Barnes, 2003). Although information about the microbiological state of South Africa's rivers is limited, the little data that is available illustrates that there is a definite need to worry. The faecal contamination of surface waters can be seen as one of the major causes of disease in South Africa (NWRS, 2004), and the implications that this may have on community health, food safety and the export status of our irrigated fresh produce is ominous (Barnes, 2003). With this in mind this study was done to determine the extent of contamination of the country's rivers and if this contamination really poses a threat to the health of the consumer and to those coming into direct contact with the water.

Data collected during this study showed the levels of faecal indicators in both the Mosselbank and the Plankenburg Rivers to be much higher than the 4 000 and 1 000 org.100mL^{-1} faecal coliforms suggested in the irrigation water guidelines by DWAF (2008) and the WHO (2006), respectively. The faecal counts in the Mosselbank and the Plankenburg rivers peaked at 460 000 org.100mL^{-1} and 160 000 org.100mL^{-1}, respectively making it unfit for irrigation purposes. Farmers are, however, forced to use this tainted water if they want to be sure of a harvest (Parrot *et al*., 2008).

The irrigation water taken from the Mosselbank site in many cases had much lower counts than that found in the river itself. This could suggest a natural "die-off" of the organisms over a period of time. If looking at the irrigated produce, however, high numbers of indicator organisms - much higher than that found in the irrigation water - was noted. This may suggest a possible build-up of organisms with the repeated application of contaminated irrigation water. Counts as high as 1 600 000 org.100mL^{-1} faecal coliforms were found on both the lettuce and the cabbage samples taken during the study, much higher than the 200 org.g^{-1} coliform organisms as indicated in the guidelines (DoH, 2006), making these products unfit for human consumption.

The potential pathogens *E. coli*, *Listeria* and *Salmonella* were present in almost all of the river water samples, in many of the irrigation samples and on several of the samples taken of the irrigated produce. The South African Department of Health states that no *E. coli* or *Listeria* should be present in one gram of a food sample, whereas *Salmonella* should be absent in a 25 g sample (DoH, 2006). As many of the irrigated samples tested positive for some of these pathogens, it would also be unfit for human consumption according to these guidelines.

Some potential pathogens including *E. aerogenes*, *E. cloacae* and *E. coli* were isolated from the river water, irrigation water and the irrigated produce, suggesting that there might be a carry-over of microbes from the irrigation water onto the irrigated produce. Accredited international methods were used for the enumeration and identification of these organisms, but to confirm that these organisms are the exact same strain molecular methods should, in future, be used for verification.

The traditional international microbiological methods were used during this study. Although specific selective media were used for the presence/absence tests of the pathogens, other colonies not described in the methods were also found on the plates. Upon investigation, these colonies often had other colony morphologies and metabolic needs than the specific organism tested for and, therefore, the question arises of how selective these media, and how accurate the API identification system actually are? This is also an aspect that must be examined in future studies.

Another explanation for this may be that these organisms are taken out of their natural habitat (the intestinal tract of mammals) and may therefore mutate to survive in their new environment. Either way this has enormous implications for the food industry as possible human pathogens may now slip by unnoticed, while other harmless organisms may mistakenly be identified as possible pathogens.

Recommendations and Future Research

From both the literature reviewed and the study on the Plankenburg and Mosselbank Rivers it is clear that these local rivers are faecally polluted to a dangerous extent. It is not normal for such high levels of potential pathogens to be present in river water and their presence indicates a serious faecal pollution problem. However, the study, especially on the Plankenburg River, was only done over a relatively short period and thus before more substantial conclusions can be reached both rivers will have to be monitored over at least two full summer and winter seasons.

To endeavour to find an explanation for the unidentifiable colonies found on internationally recommended selective media used in these methods, more in-depth research should be done with regards to the selectivity of the media and the possible mutation of organisms outside of their natural habitat.

As many of the isolated colonies from the different selective media could not be identified with the use of the API-Web system, other methods of identifying these organisms should be investigated. Before recommendations to governing bodies can be done it will also be essential to confirm that a “carry-over” of organisms do take place. For this it is recommended that molecular methods be used to positively verify that the organisms are from the same source.

REFERENCES

Barnes, J.M. (2003). *The Impact of Water Pollution from Formal and Informal Urban Developments along the Plankenbrug River on Water Quality and Health Risk*. PhD Dissertation, University of Stellenbosch, South Africa.

Beuchat, L. R. (1996). Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection*, **59**, 204–216.

DEAT (Department of Environmental Affairs and Tourism) (2006). South Africa Environment Outlook. A report on the state of the environment. Department of Environmental Affairs and Tourism, Pretoria.

DoH (Department of Health) (2006). Guidelines for environmental health officers on the interpretation of microbiological analysis data of food. Department of Health, directorate: Food Control. Annexure B: Proposed microbiological specification to be used as guidelines for foods. Government Printers: Pretoria.

DWAF (Department of Water Affairs and Forestry) (2008). Guidelines for assessing the potential health risk for the four water uses. National Microbial Monitoring Programme. Bellville, South Africa.

NWRS (National Water Resource Strategy) (2004). First Edition, September 2004. Department of Water Affairs & Forestry, South Africa.

Parrot, L., Sotamenou, J., Kamgnia, B.D & Nantchouang, A. (2008). Determinants of domestic waste input in urban agriculture lowland systems in Africa: The case of Yaounde in Cameroon. *Habitat International*, doi:10.1016/j.habitatint.2008.08.002, Pp. 1–8.

Stats SA. (2006). South African Statistics. [www document]. URL: http://www.ukzn.ac.za/heard/resources/Stats/SA%20Statistics%202006.pdf. 4 March 2009.

WESGRO (2006). Fruit processing sector brief. Western Cape Trade and Investment Promotion Agency. Pp. 36-40.

WHO (World Health Organization) (2006). *Wastewater Use in Agriculture* : Guidelines for the safe use of wastewater, excreta and greywater, Vol 2. Geneva, Switzerland.